SLV	Sapporo-like virus
SMAC	sorbitol-MacConkey agar
SPHL	State Public Health Laboratory
SRSV	small round structured virus
THM	trihalomethanes
TQM	total quality management
UV	ultraviolet
WAG	Water Action Group, Erickson, B.C.
WDOH	Wisconsin Division of Health
WFP	water filtration plant
WHO	World Health Organization
W.I.	Walkerton Inquiry
Y2K	year 2000

Those who cannot remember the past
are condemned to repeat it.
— George Santayana

1

INTRODUCTION

The death rate in the industrialized world has decreased dramatically since consistent mortality records have been kept (Figure 1.1). A key feature of this improvement has been a major reduction in the infant death rate, which is the largest single contributor to our lengthening life expectancy. Reduction in the occurrence of waterborne diseases such as cholera, typhoid and other diarrheal diseases has contributed substantially to reducing the infant death rate, with the largest incremental improvements coming during the early 1900s.

In contrast, we continue to experience a devastating toll of illness and death in many of the most populous regions of the globe. The World Health Organization (WHO) estimates that 2.1 million people die every year from diarrheal diseases (including cholera), that the majority of these deaths is among children in developing countries and that 65% of these fatalities could be prevented by water, hygiene and sanitation interventions (WHO, 2002). The focus of this book is on the ability of fecal-oral disease transmission via drinking water to render drinking water unsafe. The context of drinking water transmission within the whole range of fecal-oral disease transmission is shown in Figure 1.2.

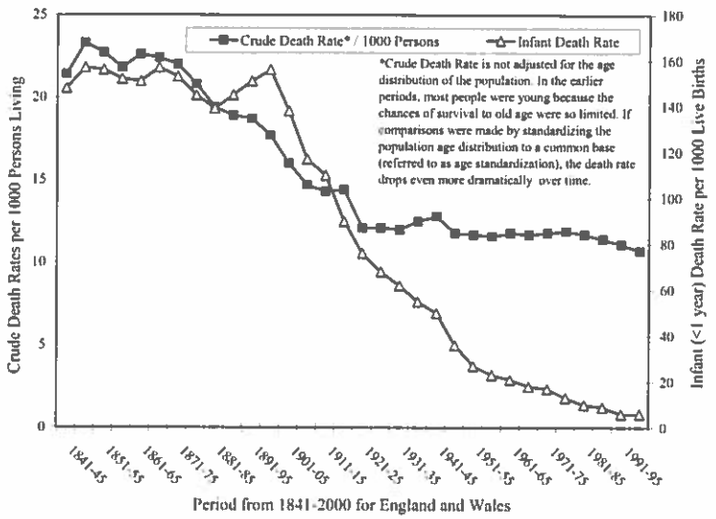

Figure 1.1 Reduction in death rates since comparable records have been maintained for England and Wales (Source: Office for National Statistics. Crown copyright material is reproduced with the permission of the Controller of HMSO and Queen's Printer for Scotland)

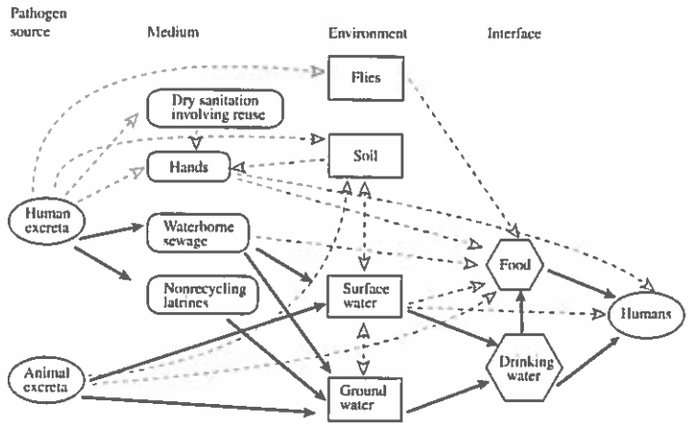

Figure 1.2 Drinking water disease transmission (solid lines) among fecal-oral disease pathways (adapted from Prüss et al., 2002)

The stark contrast between the experience in industrialized countries and that in the rest of the world demonstrates the enormous benefit that is achieved by implementing and maintaining effective practices for producing safe drinking water. Unfortunately, success can breed complacency. We have had and continue to have serious outbreaks of waterborne disease in affluent nations. Reviewing the cases presented in this book reveals in hindsight that these outbreaks were preventable. Similar outbreaks should not reoccur if we learn the lessons these cases have to offer. Perhaps more disturbing is the number of cases that were eminently preventable with even a minimal degree of foresight (e.g., Walkerton and North Battleford). The challenge is to convert hindsight into foresight.

In fairness to those involved in these failures, the means for prevention are always more obvious in hindsight. Reading the detailed accounts revealed at the Walkerton (O'Connor, 2002a) and North Battleford (Laing, 2002) Inquiries is disturbing because some of the oversights or failures described could conceivably happen to most people, on a bad day. Problems arise when small individual mistakes are allowed to accumulate; elements of failure gradually evolve into dangerous circumstances without revealing a clear warning of the scope of emerging disaster.

Although the literature is filled with reports of waterborne outbreaks, in a number of cases these accounts do not appear to have reached the consciousness of enough of those who are responsible for providing drinking water. Even the overwhelming publicity about the Walkerton tragedy in Canada was insufficient to prevent another major Canadian outbreak 11 months later in North Battleford: many of the same elements of failure contributed to the later outbreak.

These revelations are not recent. Consider the following quote from Edwin C. Lippy:

> The disturbing trend toward more frequent occurrence of waterborne disease outbreaks should serve as a warning to all who share in the responsibility for the delivery of a safe and potable water. The 'multiple barrier' concept that relies on placing protective systems between the water consumer and actual as well as potential sources of contamination should be emphasized, with appropriate consideration for natural features (distance, dilution, geologic factors), man-made facilities (adequate waste treatment, water treatment, operational considerations) and conscientious surveillance by regulatory agencies (monitoring, inspection, certification).

This statement could have been written, with little modification, as an overview commentary for either of the Walkerton or North Battleford Inquiry reports; it was published in 1981 concerning the investigations of three U.S. outbreaks in 1979 (Lippy, 1981).

The number of things that can be wrong or go wrong without having an outbreak is both striking and troubling. The positive news is that a multiple barrier approach makes an outbreak much less likely. Some may argue that affluent societies are

chasing diminishing returns by invoking more stringent measures to prevent outbreaks that are already infrequent. Certainly there are many environmental health issues where the pursuit of trivially small risks can be futile (Hrudey & Leiss 2003). However, the source of contamination that causes waterborne outbreaks of infectious disease is present wherever humans reside: human or animal fecal wastes. With this source of contamination, known to be capable of causing disease and death, always nearby, our practices to maintain the safety of our drinking water supply must be consistently rigorous.

Even with sophisticated management systems in place, water remains a very low-cost commodity. Failing to invest the relatively modest amounts that can achieve important improvements in safety is surely a false economy in an affluent society (Hrudey & Hrudey, 2002). The Walkerton Inquiry provided estimates of the costs for implementing all 93 recommendations of the Part 2 Inquiry report, which addressed the provision of safe drinking water for all of Ontario (O'Connor 2002b). These estimates identified an incremental cost (based on 10-year amortization of one-time costs) of between $7 and $19 per household per year. These are not onerous costs for assuring residents of Canada's wealthiest province that their drinking water is safe.

If our goal is to provide safe drinking water, we need to consider what is *safe*. First, let us consider what safe is not. Safe does not mean zero risk. There is no sharp line between safe and unsafe. Safe is not an absolute condition; it has meaning on a relative basis. Each person has a notion of safety that may vary from one risk to another. A pragmatic notion of safety is a level of risk so small that a reasonable, well-informed individual need not be concerned about it, nor find any rational basis to change his/her behaviour to avoid a negligible but non-zero risk. (Hrudey & Krewski, 1995).

In the context of drinking water, given our current capability for reducing risk, *safe* drinking water should mean that we do not expect to die or become seriously ill from drinking or using our tap water. Assuring that drinking water is essentially free (to negligible levels) from the risk of infectious disease can be and largely has been achieved for most public water supplies in affluent nations. The challenge is to maintain and extend that achievement as widely as possible. An equivalent pragmatic concept of safety was adopted by the Walkerton Inquiry (O'Connor, 2002b, p. 74).

The case studies reviewed for this book demonstrate that a zero risk of a waterborne outbreak can never be achieved; failures can happen in too many ways to assure zero risk. Humans inevitably make errors: there is little value in continuing to characterize failures as being caused by human error when we know that human errors are inevitable (Kletz, 2001). Rather, the focus must be on designing and maintaining systems that are able to preserve safe outcomes when human errors do occur.

No single action can be implemented to prevent outbreaks; there is no magic. Based on the cases presented here, we conclude that prevention can best be achieved

by employing informed individuals committed to providing safe water. Hopefully, providing access to this compilation of the details from so many outbreak failures will equip committed people with a better appreciation of the range of things that can and do go wrong. In addition to raising awareness, these details should provide evidence of the need to take sensible actions when drinking water safety is at risk to convince those bottom-line-driven skeptics who may promote short-sighted false economies in the name of cost-saving or efficiency.

Converting hindsight into foresight is essentially an attempt to avoid having to learn painful lessons the hard way. By sharing the accounts of the problems and characteristics of these outbreak case studies, we hope to provide anyone who wishes to prevent drinking water outbreaks with a tangible sense of what can go wrong and why. If we can initiate and implement the conceptual equivalent of defensive driver training — learning how to avoid accidents without having to experience them — this book will achieve its primary objective.

Figure 1.3 Walkerton Memorial (photo by S.E. Hrudey)

2

CAUSES OF WATERBORNE DISEASE OUTBREAKS

2.1 HISTORICAL BACKGROUND

Clean water has always been vitally important to human health and well-being, but the scientific understanding of how infectious disease can be spread by microscopic disease-causing agents (pathogens) in water has only been established over the past 150 years. The history of the understanding of water's role in disease transmission is rich, fascinating and complex. By introducing a few key details of the pioneering discoveries, we hope to convey some of the broader themes that also recur among many of the case studies that form the heart of this book.

Dr. John Snow (Snow, 1849) was the first to publish a rigorous explanation of how cholera was spread by sewage-contaminated drinking water in England. Snow's work was only one of a long series of publications in the British health and medical literature (Smith, 2002) attempting to explain the causes and transmission mode of cholera, which first reached Britain in the fall of 1831 and caused more than 21,000 deaths the following year (Morris, 1976). The *Lancet* devoted 44 pages of its November 19, 1831 issue to this new scourge, concluding (Lancet, 1831;

Smith, 2002): "*We can only suppose the existence of a poison which progresses independently of the wind, of the soil, of all conditions of the air, and of the barrier of the sea; in short, one that makes mankind the chief agent for its dissemination.*"

The re-appearance of cholera in Britain in 1848 and then in America in 1849 caused a renewed interest in the causes of cholera epidemics, including an insightful analysis of the global patterns of cholera outbreaks (Dickson, 1849). In the British Isles, the epidemic began in Scotland in October 1848 and appeared in London in February 1849 (Snow, 2002). This proved to be the most devastating of all the 19th-century outbreaks, registering more than 6,000 deaths in Scotland and more than 53,000 deaths in England and Wales and accounting for more than 12% of all deaths in 1849 (Morris, 1976). According to the Surgeon-General's Catalogue, these recurring disasters led 777 individual writings on the subject of cholera to be published in London between 1845 and 1856 (Pelling, 1978).

Snow's first comprehensive explanation of his analyses of the causes of cholera (Snow, 1849), if somewhat premature by his own admission, was followed into print within a month by a monograph on the same subject from physician William Budd of the Bristol Infirmary (Budd, 1849; Smith, 2002). Budd concluded:

> 1. That the cause of malignant cholera is a living organism of distinct species.
>
> 2. That this organism — in shapes hereafter to be described — is taken by the act of swallowing into the intestinal canal, and there becomes infinitely multiplied by the self-propagation, which is characteristic of living beings.
>
> 3. That the presence and propagation of these organisms in the intestinal canal, and the action they exert, are the cause of the peculiar flux which is characteristic of malignant cholera; and which, taken with its consequences, immediate and remote, constitutes the disease.
>
> 4. That the new organisms are developed only in the human intestine.
>
> 5. That these organisms are disseminated through society, (1) in the air, in the form of impalpable particles; (2) in contact with articles of food; and (3) and principally, in the drinking-water of infected places.

Budd did acknowledge the "*ingenious*" and prior findings of Snow in his own work, but he concluded that Snow had failed to identify the agent of cholera, having identified only the drinking water vehicle, on which he agreed with Snow (Smith 2002). Snow in turn cited the work of Budd, disagreeing mainly with Budd's attribution of airborne transmission as offering even a secondary transmission route. However, Snow was concerned that he be credited with priority for the discovery of the waterborne theory so he exerted some effort to ensure this view of history prevailed (Snow, 1856; Smith, 2002).

Budd, although less well-known than Snow, was no less prolific in writing about the causes of waterborne disease. In particular, he wrote extensively in the *Lancet* (Budd, 1856) and the *British Medical Journal* about the waterborne cause of typhoid

fever, a disease that was to exert a more pervasive fatal impact on industrialized countries in Europe and North America well into the 1900s.

After the 1848 and 1849 outbreaks, others investigating cholera for public health authorities contributed important evidence that was included in a report of the General Board of Health (Chadwick & Smith, 1850; Smith, 2002). Notably, Dr. John Sutherland, an inspector for the Board, working for the famous public health and sanitation advocate, Sir Edwin Chadwick, studied an epidemic associated with contaminated pump water in Salford (Sutherland, 1850). Sutherland's investigative report included a number of insightful comments from residents, including "*he was afraid of using the pump-water, on account of the water in which the bedding of two persons who had died of cholera had been washed having been thrown into the gutter and he thought it ran into the well.*"

Sutherland observed in relation to these local concerns that "*It appears that the well had been repaired, and from some cause or other, a sewer which passes within 9 inches of the edge of it had become obstructed and leaked into the well.*" Sutherland concluded that there was a remarkably strong association between household water and cholera, and that with respect to water from wells contaminated by sewage that the "*predisposition occasioned by the continued use of such water is perhaps the most fatal of all.*" Although Sutherland held allegiance to the dominant theory that cholera was spread by "*miasma*" associated with filth and bad odour — a view strongly held by Chadwick — Sutherland's 1850 report was actually closer to the view espoused by Snow and Budd.

Despite the obvious connections among cholera, fecal waste disposal and drinking water contamination, Sutherland's chief point of disagreement with Snow and Budd was the interpretation of water being some kind of "*predisposing factor*" for cholera (Sutherland) rather than being the physical vehicle for transmitting the disease-causing agent (Snow and Budd). These have come to be characterized as the "*miasma*" versus the "*contagion*" theories. Although they disagreed strenuously at the time, both sides to this debate apprehended elements of the true causal mechanisms. That is, upon exposure to and infection with the agent responsible for cholera, the causal agent proliferated in the intestinal tract of cholera victims and subsequently contaminated drinking water through the fecal discharge in the profuse, watery diarrhea characteristic of cholera.

Others were drawing conclusions about a role for water, including John Lea in the U.S. who had concluded that use of rain water or boiled water protected against cholera (Smith, 2002). He communicated these views to the British government (Lea, 1851) and applied his theories to gathering rudimentary epidemiologic evidence for a cholera outbreak in Cincinnati in 1849 and reviewing an investigation by S.O. Butler of a similar investigation in St. Louis the same year (Smith, 2002). However, Lea incorrectly believed that the mineral content of water determined its "*predisposition*" to cholera.

The 1854 cholera outbreak in London allowed Snow to extend his initial monograph with a second, greatly expanded edition that reported on two compelling

new investigations (Snow, 1855). The first investigation is, perhaps, the most commonly cited contribution from Snow, focusing on the explosive cholera outbreak that occurred in the Soho district of central London in early September 1854 (Snow, 2002). He described "*the most terrible outbreak of cholera which ever occurred in this kingdom.*" The outbreak claimed over 500 lives in only 10 days and was ultimately linked to the water supplied by the Broad Street pump, now immortalized by a monument near the John Snow pub in Soho. This happened in an area less than 10 minutes walk from his home in Picadilly. Snow undertook an analysis of the cholera deaths during the week ending September 2 and, following detailed enquiries about the source of drinking water for each case, concluded that 83% of the victims had routinely consumed water from the Broad Street pump. Snow addressed the Board of Guardians of St. James parish on September 7 with his evidence and convinced them to remove the pump handle on September 8 (Snow, 2002). The relationship of this powerfully symbolic action to the course of the cholera outbreak (Figure 2.1), based on Snow's own data reporting date of onset, shows that the outbreak was largely over by the time the pump handle was removed. The decline in cases before September 8 may have been attributable primarily to the rate at which fearful residents abandoned the cholera-stricken neighbourhood.

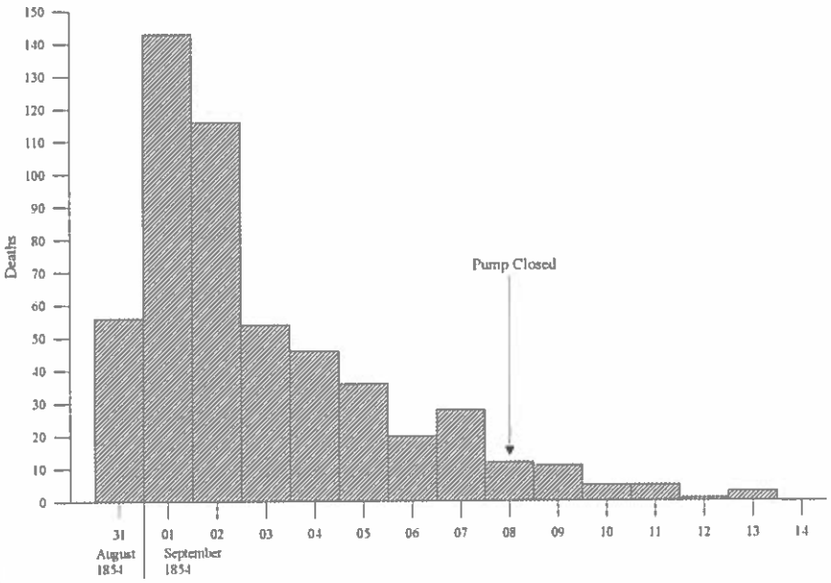

Figure 2.1 Fatal cases during the cholera outbreak in Golden Square, Broad Street, London in 1854 (Source: Snow, 1855)

Perhaps the most conclusive evidence revealed in Snow's investigation of this lethal outbreak was the finding that one of the cholera fatalities was a widow who lived in Hampstead (~7 km from Soho). She regularly received drinking water delivered from the Broad Street pump because she preferred its taste. Her last batch of water was delivered on August 31, and she died of cholera on September 2 after having consumed the Broad Street pump water (Snow, 2002). Greater credibility came to be associated with this evidence when Edwin Lankester, a fellow member of the Medical Society of London, established a Cholera Inquiry Committee to investigate the outbreak. Snow served on this committee with Reverend Henry Whitehead, curate of St. Luke's. Whitehead's follow-up investigation attributed the source of cholera infection to diaper wastes from an infant who died of cholera being disposed in a cesspool at No. 40 Broad Street (Cosgrove, 1909; Snow, 2002). Mr. J. York, the surveyor for the Cholera Inquiry Committee, was assigned the unenviable task of investigating the potential for contamination from this cesspool and its main drain, located less than 1 m from the shallow well supplying the Broad Street pump. York concluded that the crumbled, decaying brickwork he uncovered was essentially a sieve, allowing percolation from the cesspool and sewage drain into the well serving the Broad Street pump (Cosgrove, 1909). The housing and sanitation conditions in London at this time were certainly ideal for the spread of waterborne disease (Figure 2.2).

Snow's next major contribution followed his meticulous analysis of a massive natural experiment. An increasing number of London residents were having water supplied directly to their homes by pipe distribution from two different water companies. The Southwark & Vauxhall Company was drawing polluted water from the Thames River in central London near the Battersea fields, where it was affected by sewage discharges into the Thames. The Lambeth Water Company, in contrast, had moved its intake upstream of the sewage pollution of London to Thames Ditton in 1852 (Snow, 2002).

Snow's insightful analysis (Table 2.1) is commonly credited with creating the method of epidemiology, the discipline that now guides all population-based human health studies, although it is clear that he refined and effectively applied the statistical approach that was developing in the hands of several health investigators of his day. In particular, the use of death rate per household revealed the 8.5-times higher death rate for those using the more contaminated Thames River supply.

Snow's "*water is the cause*" position was overstated in relation to his actual belief in the fecal-oral route of gastrointestinal disease transmission (Smith, 2002). He said "*I believe no one ever supposed that impure water was the sole cause of cholera, and for my part, I do not consider it a cause at all, but only a frequent medium or vehicle of the true cause of the disease, namely, the reproductive cholera poison*" (Snow, 1851).

Figure 2.2 Open sewer running under lodging house in Field Lane, London. Reprinted with permission of the Guildhall Library, Corporation of London

	Number of houses.	Deaths from Cholera.	Deaths in each 10,000 houses.
Southwark and Vauxhall Company	40,046	1,263	315
Lambeth Company . . .	26,107	98	37
Rest of London	256,423	1,422	59

Table 2.1 Cholera deaths in 1854 in Snow's Grand Experiment (Source: Snow, 1855)

Sutherland and his superior, Edwin Chadwick, steadfastly defended the miasma theory — the premise that unsanitary conditions (poor living conditions, dissipated lifestyles, overcrowding and foul air) caused epidemic disease in urban populations (Halliday, 2002). Considering the persuasive statistics that

Chadwick and Sutherland produced to demonstrate an association between water source and cholera, their opposition to Snow's account appears more than a little stubborn (Hamlin, 2002). Their propensity was to overlook the compelling statistics presented by Snow in support of his contagion theory. Their own statistical analyses were also compelling if these miasma advocates had been more open to an alternate interpretation. The miasma advocates noted that contaminated water was widespread throughout England. They reasoned that if contaminated water was the primary cause, why was cholera present only intermittently, in the form of epidemics? Why was cholera not constantly present, given the common delivery of contaminated water? The answer to their dilemma lies partly in recognizing the existence and role of the causative pathogen and the balance model depicted in Figure 2.3. Only when a complex combination of circumstances occurs, including the presence of sufficient numbers of causative agents (pathogens), an effective environmental route of transmission and a number of sufficiently susceptible hosts available to be exposed, does clinical disease appear as an epidemic.

For almost 30 years, the debate about water being the vehicle that transmitted cholera continued to rage until 1883 when Robert Koch re-discovered *Vibrio cholerae*, the bacterial pathogen that causes cholera. Ironically, this re-discovery followed the little-known initial discovery of the cholera bacterium by Italian microscopist Fillipo Pacini in 1854, during the period when these debates about the causes of cholera were initiated (Frerichs, 2000). Central to the raging debates, with the main combatants holding more common ground than they realized, was the issue of predisposition, including a central role for odour versus water as a vehicle for the "undiscovered" pathogenic agent.

These concepts have developed the foundations for our current appreciation that disease results from an imbalance in the relationships among a causative agent (the pathogenic microorganism), the human host and the environment (Figure 2.3).

A bizarre experiment in Germany illustrates the intensity of the scientific debates about the causes of cholera at the time (Navashin & Piso, 1974). Max von Pettenkofer, a distinguished German medicinal chemist (e.g., he discovered creatinine in human urine) and a pioneering professor of hygiene, studied ten cholera outbreaks over 40 years, including one in Munich in which he and his daughter were stricken and their cook died. Professor von Pettenkofer concluded that cholera was not caused strictly by a miasma. Rather, he accepted a role for water within his "x" human activity factor, which had to combine with his theoretical soil-bound "y" factor to yield "z," his true cholera poison.

In 1883, when fellow German scientist Robert Koch announced his discovery of the bacteria associated with cholera, Professor von Pettenkofer accepted Koch's vibrio as the "x" factor in water, but he steadfastly maintained that it must be present together with his "y" factor to cause cholera. To prove his theory, in 1892 after the Hamburg waterborne cholera epidemic had killed 8,000, the 74-year-old professor swallowed 1 mL of recently cultured *V. cholerae* at a public meeting. He suffered

diarrhea and excreted *V. cholerae* for several days, but otherwise classified his reaction as "negative," thereby proving his theory that Koch's vibrio was insufficient to cause cholera. This remarkable, if foolhardy, demonstration of the benefits of acquired immunity served to bolster his supporters, but failed to dissuade the growing numbers of believers in the transmission role of drinking water for the causal pathogen *V. cholerae*.

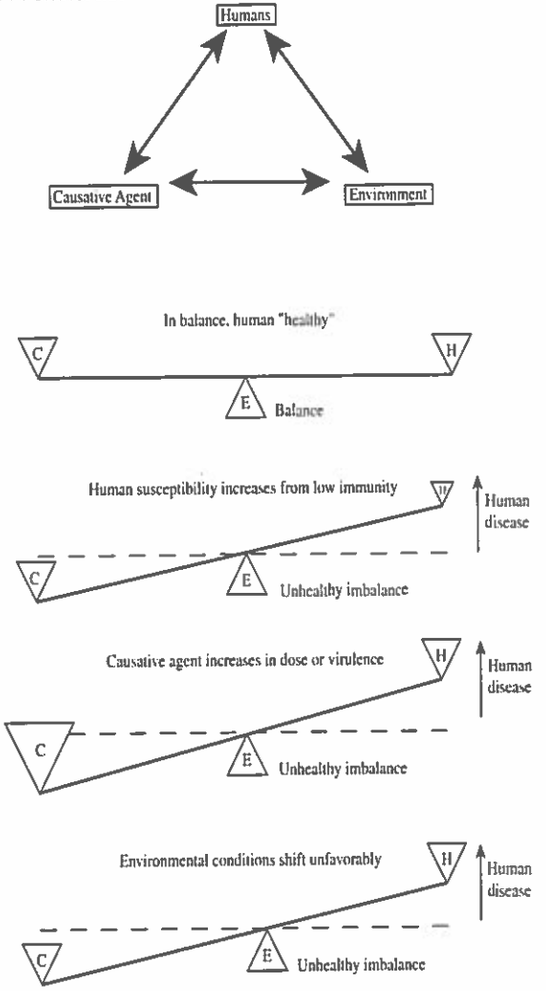

Figure 2.3 Balance among human hosts of disease, the causative agent and the human environment allowing exposure (adapted from E.T. Chanlett, *Environmental Protection*. ©1973, with permission of The McGraw-Hill Companies)

Of course, the irony of these vigorous debates is that both sides of the argument had some merit. We now recognize that a narrow view of the means for preventing waterborne disease with a strict focus on the pathogen is not adequate. A more ecological perspective of infectious disease, one that considers the interplay among the pathogen, the human host and the social and physical environment, has been advocated (Wilson, 1995). As suggested by Figure 2.3, the population burden of infectious disease involves a complex interplay among changes in abundance, virulence or transmissibility of the microbial pathogen; an increase in probability or effectiveness of exposure to the pathogen; and an increase in host vulnerability to the infection or to the consequences of the infection.

The potential for human exposure to the pathogens responsible for causing enteric disease is ever-present because of the proximity of humans to sources of fecal waste: themselves, other humans and, in the case of zoonotic hosts, pathogen-carrying animals. When a balance exists between humans and their exposure to pathogens, no disease may be evident. This balance can be disrupted in several ways. Environmental conditions (e.g., heavy rainfall or runoff) may allow greater exposure to pathogens, giving rise to a sufficient dose and resulting in disease. The causative agent may become more important because of an increased source of pathogen dose (e.g., a sewage spill) or increased virulence of the pathogens (e.g., mutation to create a more virulent strain). Either way, a factor that increases the importance of the pathogen relative to human host defenses can lead to imbalance and disease. Alternately, exposure of humans with low immunity (e.g., infants, individuals with immune disorders or those receiving immune-suppression medication) can shift the balance and increase disease.

The interactions are complex. The pattern depicted by Figure 2.4, showing the death rates from typhoid fever, another disease that was largely transmitted by drinking water, for the City of Chicago between 1860 and 1942, illustrates the complexity. The standard premise, often repeated during the training of public health and environmental engineering professionals, has been that drinking water disinfection, primarily by chlorination, can be credited with the virtual elimination of waterborne transmission of typhoid in affluent nations.

That drinking water transmission of typhoid has now been virtually eliminated in affluent nations is well established. The surprise suggested by Figure 2.4 is that chlorination, in this circumstance, appears to have played a minor role, compared with other factors. This is not the primary message that should be drawn. Rather, the message should be that major events (e.g., equipment failures, storms, disasters) and physical factors (e.g., intake location moved away from sewage discharge, diversion of sewage to another watershed) play an important part in infectious disease transmission. These factors could be expected to disrupt the balance conceived in the model of Figure 2.3.

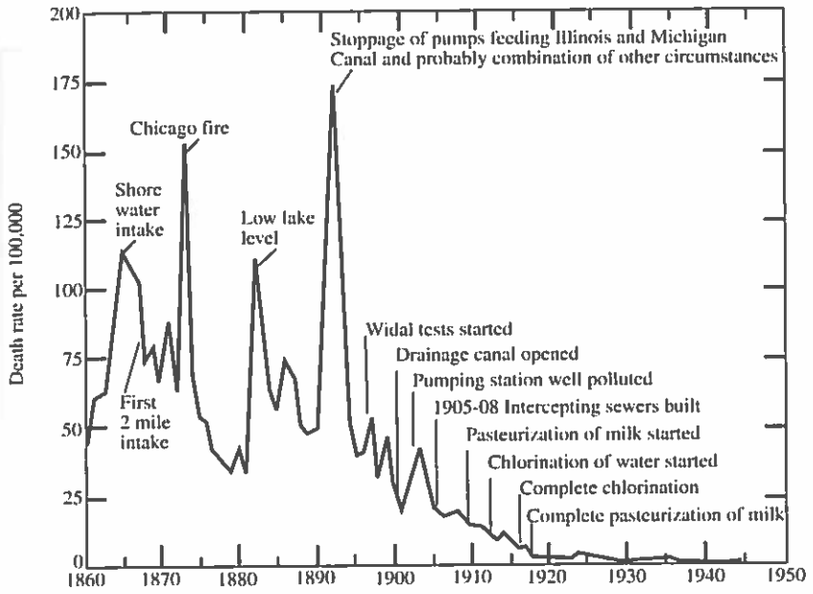

Figure 2.4 Death rates from typhoid fever per 100,000 total population, City of Chicago from 1860 to 1942 (Source: L.I. Dublin et al., *Length of Life*, ©1949, The Ronald Press)

For a chlorine-susceptible pathogen like *Salmonella typhi* (the cause of typhoid), earlier implementation of chlorination for public drinking water supplies would likely have prevented many of the epidemic peaks evident in Figure 2.4. However, success in the control of typhoid was not achieved by reliance on chlorination alone. Many other improvements in wastewater management, the implementation of drinking water filtration for surface sources (Logsdon & Lippy, 1982) and the general improvement in sanitation that occurred over preceding years, all contributed to the virtual elimination of typhoid fever transmission by drinking water that can now be claimed for the City of Chicago and other U.S. cities (Figure 2.5).

However, a review of waterborne outbreaks in North America for the period from 1920 to 1936 (Gorman & Wolman, 1938), long after chlorination had been widely implemented for drinking water disinfection in North America, found that 16,000 cases of waterborne typhoid fever were still recorded. As well, the Milwaukee typhoid outbreak in February 1916, with 500 cases and 60 deaths (among 25,000 to 100,000 cases of gastroenteritis), occurred because Milwaukee relied on chlorination alone without any other treatment (Schwada, 1934). A shutdown of chlorination for several hours (the only treatment barrier at that time) caused this massive outbreak. We will return to Milwaukee in the case studies of Chapter 4.

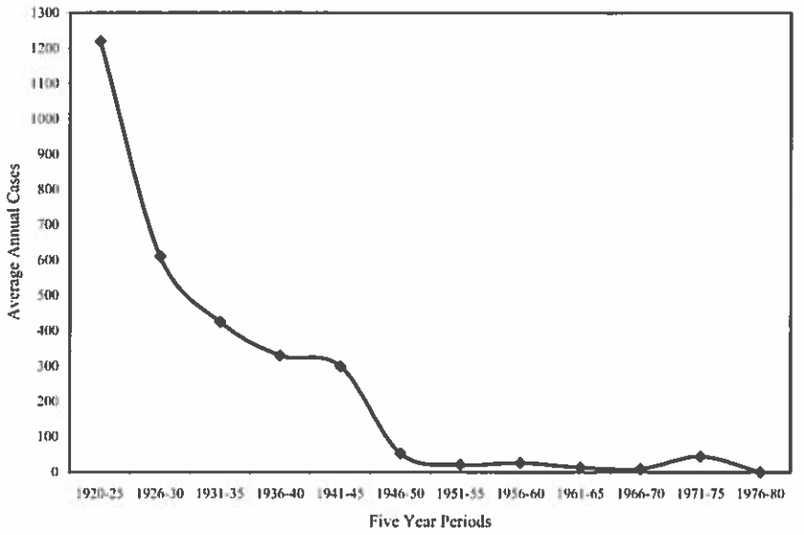

Figure 2.5 Average annual number of cases of typhoid fever occurring in waterborne outbreaks in the U.S. 1920–1980 (Source: G.F. Craun, *Waterborne Diseases in the United States*, ©1986 with permission CRC Press, Boca Raton, Florida)

The incremental benefits of multiple strategies for improving public health and drinking water safety are revisited in the Chapter 6 discussion of the multiple barrier concept. The concept of maintaining multiple levels of protection to assure drinking water safety has been advocated from the earliest days of public health engineering practice. Some politicians have questioned the short-term economic justification for making any additional investments in sanitation with some arguing that multiple barriers create unnecessary redundancy. However, the impact of widespread implementation of water treatment based on multiple barriers has dramatically reduced deaths from all drinking water outbreaks (Figure 2.6).

In 1937, a typhoid outbreak in Croydon, England caused 341 cases and 43 deaths (Galbraith et al., 1987). A public inquiry into the 1937 outbreak determined that it was caused by contamination arising while maintenance men, one of whom was confirmed as a typhoid carrier, were working in the well that was implicated as the outbreak source. The groundwater from this well was distributed unfiltered and unchlorinated; this was a case of providing no barriers beyond whatever groundwater source protection might have been in place.

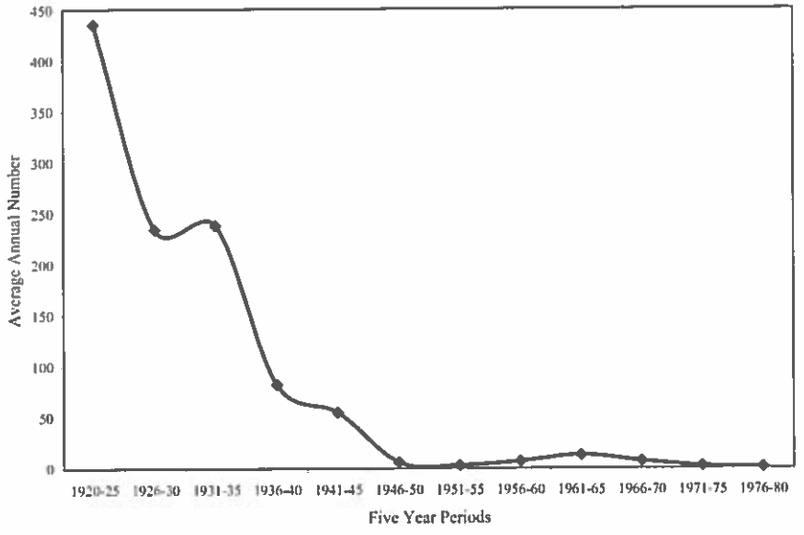

Figure 2.6 Number of deaths (per 5 years) from waterborne outbreaks in the U.S. 1920–1980 (87% from typhoid fever) (Source: G.F. Craun, *Waterborne Diseases in the United States*, ©1986 with permission CRC Press, Boca Raton, Florida)

A review of waterborne outbreaks of typhoid fever in England and Wales (Figure 2.7) showed there were 16 recorded outbreaks caused by public water supplies in the 25 years before the Croydon outbreak, but only one attributed to public water supplies in the 50 years following. Although no explanation was offered for the 1941 peak, the conditions of the war underway were presumably a factor. Following the Croydon outbreak, the occurrence of typhoid fever dropped sharply, from several thousand cases per year in the early part of the century to fewer than 300 per year in the 1980s.

During the 50-year period considered, the total number of waterborne outbreaks from all causes was highest in the last decade reviewed, 1977–86 (Galbraith et al., 1987). This apparent anomaly of having more outbreaks in recent years was attributed to improved surveillance and investigation of outbreaks revealing non-typhoid outbreaks that may have been missed in the past. Over the same period, the opportunity for sewage contamination of drinking water to spread typhoid was decreased by the lower population burden of typhoid and better overall water treatment practices.

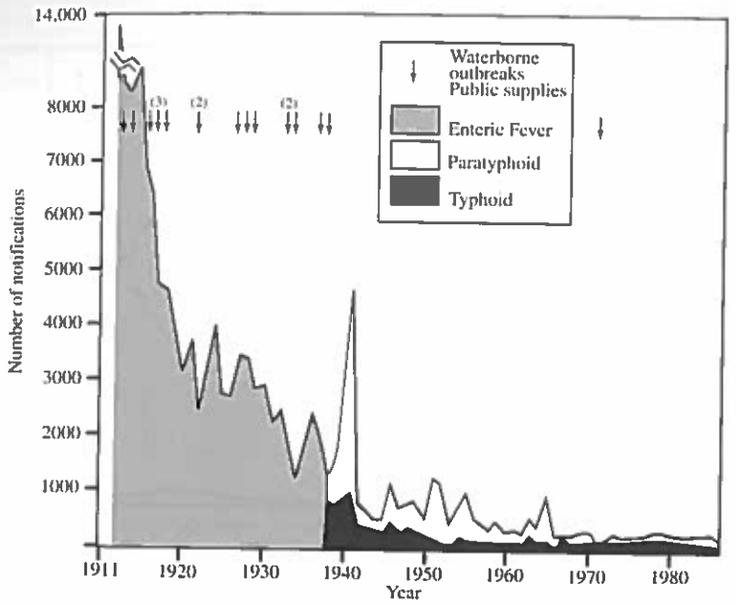

Figure 2.7 Enteric fever (typhoid and paratyphoid fevers) cases in England and Wales 1911–1986 (Source: Galbraith et al., 1987, by permission of the Chartered Institution of Water and Environmental Management)

The detection and reporting of outbreaks is not always consistent. If the balance depicted in Figure 2.3 is only slightly out of equilibrium, there may be some low-level prevalence of human disease in the community, but unless a severe imbalance occurs, the rate of disease may not be sufficient to be detected as an outbreak by public health surveillance systems. Even when disease rates are higher, disease reporting rates may be too low to identify an outbreak. This tip-of-the-iceberg phenomenon is depicted by Figure 2.8, which shows that only when an outbreak of sufficient severity occurs, relative to background disease rates, will public health authorities recognize it as an outbreak. Subsequent investigation may reveal an exposure (e.g., drinking water source, food item, personal contact) that is shared by the cases, allowing the probable source of infection to be identified. Below this detection threshold, a drinking water system may be transmitting endemic disease, but it will not be recognized or attributed to drinking water.

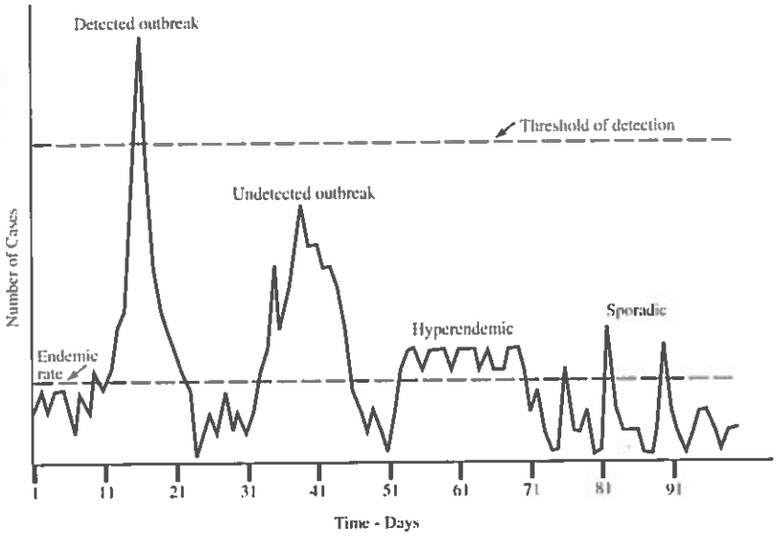

Figure 2.8 Limits to outbreak detection (Frost et al., 1996. Reprinted from *Journal AWWA*, Vol. 88, No. 9 (September 1996), by permission. Copyright ©1996, American Water Works Association)

The history of the understanding of water's role in disease transmission reveals some common themes about the investigation of waterborne outbreaks and the attribution of their causes to specific agents. These include the need for:

- determined and focused investigation to reveal the underlying factors and direct causes of any outbreak;
- evidence from various disciplines to understand the complex, interacting processes involved in causing an outbreak;
- opinions and arguments from among a number of individuals with varying perspectives, expertise and approach to sort through conflicting and unavoidably incomplete evidence;
- generation, consideration and resolution of multiple competing theories to develop the most robust explanation of what happened;
- recognition that most outbreaks will involve the convergence or alignment of several contributing and causative factors to create the outbreak conditions; and
- extraction of the key lessons about the causes of an outbreak to provide information for better preventive measures in the future.

2.2 MAJOR CLASSES OF WATERBORNE PATHOGENS

Microbial agents that cause disease are commonly referred to as pathogens. For the purposes of this book, we will focus on three classes of microbial agents that have been involved in the case studies: viruses, bacteria and protozoa. Protozoan pathogens may also often be referred to as parasites.

The major characteristics of these classes of microorganisms are outlined below, along with the pathogens from each class considered in this book. However, we must not assume that these are the only pathogens of concern in affluent nations, nor do they represent the full range of important pathogens for waterborne disease worldwide. Those interested in comprehensive coverage of waterborne diseases worldwide are referred to the excellent book by Hunter (1997). Likewise, those seeking an extensive summary of waterborne pathogens are referred to the excellent AWWA (1999a) manual and a new book (Cloete et al., 2004) on this subject.

The matter of "emerging pathogens" is also a concern (WHO, 2003a). Table 2.2 shows the number of waterborne pathogens identified since 1972, five of which are involved in the case studies that follow. These pathogens may be considered to be "*emerging*" because only recently have the tools to identify them been developed, even though some may have been causing human disease for many years without that disease being attributed to these specific pathogens.

A humorous perspective of the nature of microbes found in London's water in 1850 is shown in Figure 2.9.

Figure 2.9 An 1850s view of what could be seen in a drop of London water under a microscope (Source: Punch, 1850)

Table 2.2 Major waterborne pathogens causing diarrheal disease identified since 1972 (Source: extracted from Desselberger, 2000, with permission. Copyright ©1990 from The British Infection Society)

Year Identified	Pathogen	Comments on disease
1972	small round structured viruses (SRSVs, calciviruses)	rapid onset, acute diarrhea, often associated with vomiting in younger patients
1973	Rotaviruses	infantile diarrhea, often endemic in developing world
1976	*Cryptosporidium parvum*	profuse watery diarrhea, important for waterborne outbreaks because of resistance to chlorine disinfection
1977	*Campylobacter* spp.	acute diarrhea, sometimes bloody
1983	*Escherichia coli* O157:H7	acute, often bloody, diarrhea, hemolytic uremic syndrome (HUS)
1992	*Vibrio cholerae* O139:H7	new strain of epidemic cholera

2.2.1 Viruses

Viruses are the smallest and simplest forms of infectious agents that are able to replicate and thereby qualify as life forms, with the possible exception of prions, the as-yet poorly understood proteins implicated in causing bovine spongiform encephalopathy (BSE or mad cow disease). Viruses carry their genetic material as single- or double-stranded DNA or RNA. Viruses are not equipped to reproduce in isolation, but must infect a living cell and take over its genetic machinery, substituting their own genetic material for that of the host cell. With this substitution in effect, the invaded cell becomes a factory for making copies of the invading virus, which are then released to invade and subvert other living cells within the infected host. This microscopic pyramid scheme allows the infectious virus to affect the health of a whole organism by invading and damaging a large number of the host's cells, usually within a specific tissue or organ. The host organism's response to this invasion, by means of its immune system, will often give rise to symptoms such as fever and inflammation that typically accompany an infection. Viruses need to use host cells to achieve reproduction; they cannot replicate as free-living organisms in water (Specter et al., 2000; Hurst et al., 2002). Because viruses are dependent organisms, they possess no means of moving themselves within water, so their transport in water is entirely passive.

The elegant simplicity and efficiency of viruses means that they can be very small, between 20 to 100 nm (0.020 and 0.10 μm). Because they require none of their own reproductive machinery, the simplest viruses consist of a protein shell surrounding genetic material. Some viruses also possess an envelope or outer layer derived from the host cell membrane in addition to their protein shell. These structures allow for a wide range of diversity among viruses, and some can mutate over their short generations to adapt rapidly to changing environmental conditions and host immune responses. This rapid evolution is most commonly found among respiratory viruses like the influenza viruses.

Because viruses are present in small numbers in the water environment, filtration on virus-adsorbing filters must be used to concentrate volumes of water ranging from 1 to over 1000 liters. Methods using various animal cell cultures have been developed and used since the 1970s to detect viruses in water. Few laboratories have the expertise to analyze water samples for viruses because this requires mastering cell culture and manipulating viruses under stringent biosafety conditions. Emerging molecular biology techniques (e.g., polymerase chain reaction, PCR) allow the sensitive detection of viral genetic material, but will not necessarily indicate the infectivity of these agents. A combination of cell culture and molecular methods is gaining acceptance because this provides information on infectivity; if the virus grows in cell culture, it is infective (Köster et al., 2003).

Physical removal of viruses by conventional water filtration (granular media) processes is limited by their size. This feature has raised renewed concern about the ability of these small particles to travel long distances in groundwater where purification depends on natural filtration through geological media (Abbaszadegan et al., 2003). Most viruses are considered moderately susceptible to disinfection by chlorination, being more resistant than common bacterial pathogens, but generally less resistant than protozoan pathogens.

The three specific examples of viral pathogens considered (Section 2.3) are hepatitis A virus, norovirus (Norwalk-like virus) and rotavirus.

2.2.2 Bacteria

Bacteria are the class of microorganisms, which include the first identifed waterborne pathogens, as recounted in Section 2.1 for cholera. Because cholera epidemics are now virtually non-existent in affluent nations, *V. cholerae* will not be considered further in this book, despite its seminal role in shaping our earliest knowledge about drinking water disease transmission and its continuing role as a pathogen of major concern in developing nations.

Bacteria are single-celled organisms with the simplest structural plan of all cellular organisms; they have no separate nucleus for holding their genetic material. These organisms exist in every ecosystem of the planet, living under

virtually every environmental condition. Bacteria, along with other microbes and scavenger organisms, play an essential role in the function of our global ecosystem by recycling nutrients within the biogeochemical cycles (e.g., carbon, nitrogen, phosphorus), keeping our ecosystem viable. Humans are inevitably and continuously exposed to bacteria. Only a small number of all bacteria are pathogens and the distinctions (from a human perspective) between beneficial and dangerous organisms are often subtle. Notable in this regard are *Escherichia coli*, which normally colonize the intestines of warm-blooded animals (including humans) where they assist the host with digestion. The vital role of these natural bacterial flora is demonstrated when a patient receives antibiotics to control an infection and experiences indigestion or diarrhea caused by the antibiotic disrupting the natural flora of the gastrointestinal tract. Despite the essential role of *E. coli* in human digestion, certain variant strains that have acquired additional genes, most notably *E. coli* O157:H7, are virulent pathogens that can cause fatal human disease.

Bacteria reproduce by binary fission, allowing astronomical numbers of bacteria to grow within an infected host when nutrient and local environmental conditions are favourable. For illustration, with a realistic generation time of 20 minutes, a single bacterium could explode to a population of ~70,000,000,000 (i.e., 2^{36}) bacteria in 12 hours (36 generations), if the nutrient conditions did not become limiting and all generations survived, factors that will normally limit the total viable numbers. Humans rely on their immune systems to keep bacteria from proliferating unchecked.

Bacteria can reproduce in water under favourable conditions. In fact, bacteria are inherently aquatic organisms even though they must often survive in water-limited environments. However, most pathogens prefer the rich environmental conditions of a host body — abundant nutrients and warm temperatures — to the cool and nutrient-deficient conditions that exist in relatively clean natural waters. Pathogenic bacteria are expected to die off, rather than multiply, in the natural environment, but the die-off rate is highly variable as a function of specific environmental conditions and individual organism coping capacity. For instance, very cold temperatures may simply preserve rather than incapacitate pathogenic bacteria, but this fact is commonly misunderstood. In most circumstances, pathogens will die off at moderate ambient temperatures when they cannot compete for limited nutrients with the natural bacterial flora that are adapted to the ambient conditions.

Bacteria typically have one of three shapes — spherical (*cocci*), rod (*bacilli*) or spiral (*spirilli*) — and range in size from 0.3 to 2 μm, depending on which axis is measured (Haas et al., 1999; Nelson et al., 2001). Bacteria have historically been classified according to a technique known as the Gram stain, with gram-positive bacteria staining blue/purple and gram-negative bacteria staining red/pink. This classification reflects different cell wall characteristics: gram-positive bacteria have much thicker cell walls composed of peptidoglycan, a complex organic polymer combining carbohydrate and protein elements. The

thinner gram-negative cell wall is covered with a complex mosaic of proteins, lipids and lipopolysaccharides (LPS) (Stryer, 1995). Lipopolysaccharides are a major factor in the virulence of gram-negative bacteria and are often implicated in allergic reactions associated with inhalation exposure to bacterial aerosols.

Bacteria often have external structures called flagella that are used for moving in water. The flagellum is typically a whip-like structure that provides a bacterium with the independent mobility that a virus lacks. The flagellum, as a protein structure, provides an antigen, which is characteristic of a given strain of bacteria. Similarly, the cell wall composition also provides a characteristic antigen that can be used for strain typing. For example with the pathogenic strain of *Escherichia coli* O157:H7, the O antigen 157 refers to the cell wall antigen and the H antigen 7 refers to the flagellum antigen.

Bacteria are somewhat easier than viruses to remove by physical processes such as filtration, but bacteria are still far too small to be removed by straining action in typical granular media filtration processes. Instead, the physical removal of bacteria in water treatment depends upon the coagulation and flocculation processes (Section 3.3.1) that cause particles to clump together and be captured by the filtration media. However, these processes alone cannot be relied upon to remove sufficient numbers of bacteria from raw water and an additional disinfection process is required. Generally, bacteria are readily susceptible to chlorine and chloramine-based disinfection processes.

The bacterial pathogens reviewed in more detail below (Section 2.3) include *Campylobacter* spp., *Escherichia coli* O157:H7, *Salmonella* spp. and *Shigella* spp.

2.2.3 Protozoa

Protozoa are single-celled organisms, but are the most complex of the waterborne pathogenic microbes considered here. Most protozoa are benign, but a small number have adapted to live off an infected host. These infectious organisms are studied in the field of medical parasitology (Pfaller & Fritsche, 1995; Nelson et al., 2001).

The protozoan single cell is more complex than the bacterial cell because it contains distinct organelles. A separate nucleus for storing genetic material is the primary feature that distinguishes protozoa from bacteria. Protozoa have a cell membrane, but lack the cell wall that is characteristic of bacteria. The reproductive cycles of protozoa are much more complex than those of either bacteria or viruses, with both asexual (fission of a single cell) and sexual (merging of separate cells) reproduction evident in some species. Some protozoa, including those of interest to this discussion, are able to form cysts (containing a single parasite) or oocysts (containing multiple parasites) when they are faced with a challenging external environment. These cysts or oocysts represent a life stage in which the protozoa develops a round or oval form with a

protective coat that resists drying, temperature change and disinfectant chemicals. The protozoa of interest to waterborne disease transmission are excreted in the feces of infected individuals and may be transported in water as cysts or oocysts. Reproduction (either sexual or asexual) occurs only when the cysts or oocysts have found a favourable environment such as within the gastrointestinal tract of a suitable host. When the protozoa emerge from their cyst or oocyst form, they develop through various active living stages that may exhibit motility (e.g., have flagella). Cysts and oocysts typically range in size from 2 to 50 μm.

The size of protozoan cysts and oocysts makes them more susceptible to removal by conventional granular media water filtration processes than either bacteria or viruses. However, these particles are still too small to be removed by the straining mechanism of such filters. Accordingly, cyst and oocyst removal depends on effective coagulation and flocculation treatment before filtration. If done correctly, such processes are effective, and a substantial number of cysts and oocysts can be removed. But cysts and oocysts are much more resistant to chemical disinfection than are either bacteria or viruses. Accordingly, drinking water treatment requirements are often determined by the need to ensure adequate removal or inactivation of *Giardia*, *Cryptosporidium* and *Toxoplasma gondii*, the protozoan parasites discussed below (Section 2.3).

2.3 SPECIFIC PATHOGENS RELEVANT TO OUTBREAK CASE STUDIES

The pathogens reviewed here are those identified in the case studies in Chapter 4. Their relevance to this book is self-evident, but we must not ignore other pathogens capable of being transmitted by drinking water. Drinking water professionals are strongly encouraged to become familiar with the more comprehensive guides on drinking water pathogens, particularly as they are updated (Hunter, 1997; AWWA, 1999a; Cloete et al., 2004).

2.3.1 Hepatitis A Virus

Hepatitis A virus (HAV) is a small, single-stranded RNA virus (~7,500 nucleotides in length) belonging to the family *Picornaviridae* (Hunter, 1997; Sobsey, 1999). The virus is a spherical (icosahedral) particle ~27 nm (0.027 μm) in diameter with no envelope. Humans are the natural and primary reservoir for HAV, but other primates can be infected. The normal route of transmission for HAV is fecal-oral, typically

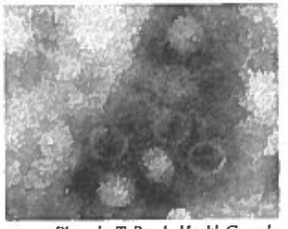

Photo by T. Booth, Health Canada

person-to-person, but transmission can occur in water. HAV is stable in water warmer than 60°C and is relatively stable in the environment, being resistant to heat, extremes of pH and microbial proteolytic enzymes naturally found in water and soil.

After ingestion, the virus passes through the stomach and begins to replicate in the lower intestine, continuing its main replication after uptake in the liver. Bile transfers the virus to the intestinal tract to contaminate feces. The viral numbers in feces peak one or two weeks before the onset of symptoms and decline rapidly after symptoms appear (Chin, 2000).

HAV causes "infectious hepatitis" with symptoms of acute inflammation of the liver (Sobsey, 1999). It is usually a mild illness, lasting one to two weeks, but it may be severely disabling with a duration of several months (Chin, 2000). The fatality rate from HAV infection is low, typically 0.1 to 0.3%, but the illness can be severe in those over 50. The disease in children is often asymptomatic, but in adults, HAV infection shows an abrupt onset of fever, malaise, appetite loss, nausea, dark urine and abdominal discomfort, typically followed by jaundice with an enlarged and tender liver. Most adults in developing countries have acquired immunity because of exposure to HAV as children, but with improved sanitation, young adults may lack immunity and may be more susceptible to outbreaks.

HAV causes at least 30,000 reported cases of hepatitis per year in the U.S., likely with substantial under-reporting (U.S. FDA, 1992; Sobsey, 1999). Although waterborne outbreaks have occurred, most cases arise from direct or indirect person-to-person transmission associated with poor housing and sanitation. In the U.S. between 1941 and 1960, there were 23 waterborne outbreaks of HAV reported with 930 cases (Craun & Calderon, 1999). From 1961 to 1970, there were 29 outbreaks reported with 896 cases. From 1971 to 1996, there were 28 outbreaks reported with 827 cases, but there were no waterborne HAV outbreaks reported in 1997–1998 (Barwick et al., 2000) or 1999–2000 (Lee et al., 2002).

HAV has been difficult to detect and confirm in water, requiring specialized and time-consuming techniques, but HAV can now be detected directly by sensitive techniques such as reverse transcription-polymerase chain reaction (RT-PCR) (Sobsey, 1999). HAV is one of the most stable enteric viruses when released in the environment, possibly persisting for months to years, particularly at low temperatures, so it can pose a risk to groundwater despite its moderate soil adsorption potential (Sobsey, 1999). A study of U.S. groundwater contamination using RT-PCR found that 31 out of 448 (6.9%) sites tested positive for HAV (Abbaszadegan et al., 2003).

The infectious dose for HAV is unknown, but has been assumed to be in the range of 10 to 100 virus particles (U.S. FDA, 1992). HAV is removed by coagulation-filtration processes to ~99% (Sobsey, 1999). Chloramine disinfection is not effective with 99.99% removal requiring disinfectant concentration-time (CT)

doses in the hundreds to thousands of mg-min/L; free chlorine is much more effective with 99.99% CT values of less than 20 mg-min/L, provided that HAV is not shielded within organic particles (Sobsey, 1999). Likewise, UV disinfection is effective as long as the virus is not shielded in particles.

2.3.2 Norovirus (Norwalk virus)

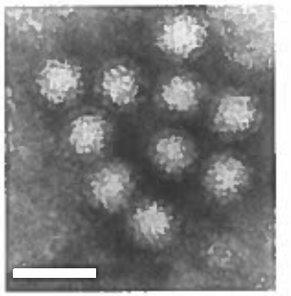

Photo by F.P. Williams, U.S. EPA
The bar is 50 nm

The Norwalk and Norwalk-like viruses (NLV) were named for the location of the first recognized outbreak in Norwalk, Ohio (Adler & Zickl, 1969; Glass et al., 2000; CDC, 2001). These pathogens are members of the family *Caliciviridae*, genus *Norovirus*. NLV have also historically been referred to as small, round, structured viruses (SRSVs). The family, now referred to as the human Caliciviruses (HuCVs), separates into four genera: *Lagovirus*, *Vesivirus*, "Norwalk-like virus" (NLV) and "Sapporo-like virus" (SLV). Only the latter two are responsible for human infections (Huffman et al., 2003). They are small, single-stranded RNA viruses with a single external protein coating that appears approximately spherical, but on closer examination has a detailed surface structure of 32 cup-shaped indentations in the shape of an icosahedron. The HuCVs, including NLV, appear to be 27 to 40 nm (0.027 to 0.04 μm) in diameter under electron microscopy (Hurst, 1999; Huffman et al., 2003).

Humans are the normal host and are the only known reservoir for these viruses (Chin, 2000). Infection is typically spread via fecal-oral transmission, following contamination of water or food or consumption of raw or lightly cooked shellfish. Infection by ingestion of food was implicated in 39%, person-to-person contact in 12% and water ingestion in 3% for 348 outbreaks attributable to NLV reported to CDC from January 1996 to November 2000; 18% could not be linked to a specific transmission mode (CDC, 2001). Approximately 96% of 90 non-bacterial gastroenteritis outbreaks reported to CDC during January 1996 to June 1997, where no other causal agent had been identified, were later found to be caused by NLV (CDC, 2001).

NLV typically produces a self-limited, mild to moderate disease with symptoms of nausea, vomiting, diarrhea, abdominal cramps, headache, low-grade fever or chills. Dehydration can cause severe consequences, including death, in susceptible persons such as the aged or those with other health conditions. Adults more commonly experience diarrhea; vomiting is more prevalent among children. Young patients may experience only vomiting (Chin, 2000; CDC, 2001). Projectile vomiting may be characteristic of the illness and may also pose a risk for transmission (Caul, 1994; Huffman et al., 2003). NLV

is very contagious and rapidly spreads from person to person, with fewer than 100 virus particles necessary for transmission (Huffman et al., 2003).

The incubation period from exposure to onset of symptoms ranges from 12 to 48 hours with symptoms typically lasting 12 to 60 hours. During this period, viruses are shed in vomitus and feces, possibly for prolonged periods after acute symptoms have subsided (CDC, 2001). Susceptibility is common — at least 50% — although many hosts show no symptoms. Immunity is short-lived, being absent 27 to 42 months after an earlier NLV illness (Chin, 2000).

These viruses can be detected with limited sensitivity by direct or immuno-electron microscopy (IEM). RT-PCR assays are the most sensitive diagnostic methods currently available (Schwab, 1999; Chin, 2000). Confidence in previously reported survival time studies for HuCVs outside of the host is limited by the current lack of tools for culturing them successfully *in vitro* (Huffman et al., 2003). Existing studies have been based on infectivity in volunteers and are not adequately quantitative, but they have shown that NLV was still able to infect volunteers after exposure to pH 2.7 for 3 hours at room temperature or incubation at 60°C for 30 min (Scwab, 1999). NLV survives freezing, allowing it to spread through ice made for human consumption, a transmission vehicle reported for some outbreaks (Cannon et al., 1991; Khan et al., 1994).

Human volunteer studies with NLV (Keswick et al., 1985) showed that substantial infectivity remained for NLV solutions dosed with 3.75 mg/L free chlorine leaving zero residual free chlorine and 1.5 to 2 mg/L total chlorine after 30 minutes of contact time. No volunteers were infected by solutions treated with an abnormally high dose, 10 mg/L of free chlorine leaving 5 to 6 mg/L residual free chlorine after 30 minutes. This study did not measure concentrations of NLV in the treated solutions so that the degree of inactivation for these treatments could not be determined (Huffman et al., 2003). NLV experienced only ~90% removal after treatment with 2 mg/L of monochloramine at pH 8 for 3 hours (Shin & Sobsey, 1998).

Removal of these viruses by natural filtration or water treatment processes has been subject to limited research (Huffman et al., 2003). Removal of non-infectious NLV in quartz sand was strongly influenced by pore water pH over the range from 5 to 7; retention of viruses by the sand filter was found to be low at pH 7 (Redman et al., 1997). A U.S. study of groundwater contamination by viruses using RT-PCR found that 3 out of 317 (0.9%) sites tested positive for NLV, but 21 out of 442 sites (4.8%) were positive for infectious enteroviruses by cell culture (Abbaszadegan et al., 2003).

2.3.3 Rotavirus

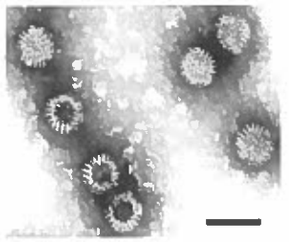

Photo by F.P. Williams, U.S. EPA
The bar is 100 nm

The rotaviruses, classified into the *Reoviridae* family, are large-structured, spherical viruses of about 70 nm (0.07 µm) diameter (Abbaszadegan, 1999; Chin, 2000). They are icosahedral, and have a double-layered protein covering, although they are not enveloped. Their genetic material consists of 11 segments of double-stranded RNA (Abbaszadegan, 1999). There are six known rotavirus groups. Groups A to F are found in animals, with Group A also common in humans, particularly infants, while Group B is uncommon in infants but has caused large adult epidemics in China. Group C is found in humans but is uncommon. The animal rotavirus groups generally do not appear to cause disease in humans, and Group B and C rotaviruses found in humans appear distinct from the animal forms (Chin, 2000).

Rotavirus produces a sporadic, seasonally occurring, often severe illness for infants and young children, involving symptoms of vomiting, abdominal distress, fever and watery diarrhea that can lead to severe dehydration causing death (Abbaszadegan, 1999; Chin, 2000). These symptoms do not normally allow rotaviral disease to be readily distinguished from acute diarrhea caused by other agents.

Rotavirus is the cause of more than one-third of hospitalized cases of diarrheal disease in infants and children under five, responsible for an estimated 3.5 million cases of diarrhea and 125 deaths among infants and young children in the United States and an estimated 600,000 to 870,000 annual deaths worldwide (Abbaszadegan, 1999; Chin, 2000). Effectively, all children are infected with rotavirus in their first 3 years of life with the highest incidence in the 6- to 24-month age group (Chin, 2000). Given the common occurrence of rotavirus infections, the more serious consequences are presumably dictated by the circumstances and severity of exposure combined with the ability of the host's immune system to defeat the infection.

Rotaviruses are transmitted by the fecal-oral route and have been implicated in at least nine documented waterborne outbreaks (Abbaszadegan, 1999), one of which is considered in the case studies presented in Chapter 4 (Hopkins et al., 1986). Rotaviruses are ubiquitous in human wastewaters (Abbaszadegan, 1999), so the conditions for waterborne rotavirus outbreaks are likely in place whenever human sewage contaminates drinking water. Widespread host immunity in the exposed population may limit the impact of such outbreaks. A U.S. study of groundwater contamination by viruses using RT-PCR found that 62 out of 448 (13.8%) sites tested positive for rotavirus (Abbaszadegan et al., 2003).

The incubation period is typically less than 48 hours, but may range from 24 to 72 hours, with the duration of illness typically in the range of 4 to 8 days

(Abbaszadegan, 1999; Chin, 2000). Huge numbers of rotavirus are excreted in the feces of infected individuals, as high as 10^{10} per g of feces (Abbaszadegan, 1999). Excretion normally ceases by about the eighth day of infection, but excretion for more than 30 days has been reported for immunocompromised patients (Chin, 2000). An estimate of infective dose is not known for rotavirus, but this is likely to be highly dependent upon the individual immune response given the ubiquity of this agent. Rotavirus was reported to have an extremely high attack rate with >40% of those exposed becoming ill in the Eagle-Vail outbreak, but actual rotavirus exposures through contaminated drinking water were not directly quantified in that outbreak (Hopkins et al., 1986).

Rotaviruses are monitored by a variety of methods including immuno-electron microscopy (IEM), radioimmunoassay (RIA) and enzyme-linked immunoassays (ELISA). IEM requires large numbers (~10^5 per mL), making it not useful for most environmental samples. Newer techniques are now based on reverse transcriptase – polymerase chain reaction (RT-PCR), providing highly sensitive monitoring techniques that need careful validation and interpretation (Abbaszadegan, 1999).

Rotaviruses are believed to resist inactivation at extreme lows or highs of pH (3.5 or 10), can survive sewage treatment and may survive for days to weeks in receiving waters, depending on water quality and temperature (Abbaszadegan, 1999). Like other enteroviruses, they are removed by conventional water treatment processes and inactivated by chlorination, although not as effectively as are enteric bacteria.

2.3.4 *Campylobacter*

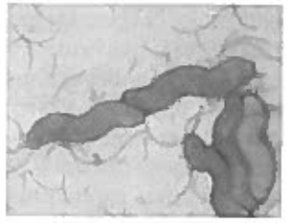

Photo by: Richard Sherburne, University of Alberta

This pathogen is a member of the family *Vibrioaceae*. *Campylobacter* is a relatively recently recognized human bacterial pathogen. Although bacteria of the genus *Campylobacter* were first isolated in 1909, it was 1977 before *Campylobacter* organisms were commonly accepted as human pathogens and *C. jejuni* was recognized as one of the leading causes of gastroenteritis in humans (Blaser et al., 1983). The genus *Campylobacter* includes 14 species, several of which are pathogenic to humans and animals (zoonotic). Most human illness is caused by *C. jejuni, C. coli* and *C. upsaliensis* (Fricker, 1999). They are gram-negative curved rods, 0.2 to 0.5 µm wide and 0.5 to 5.0 µm long with a single flagellum for motility.

Campylobacter spp. maintain a wide range of animal hosts, including domestic and wild animals. Birds provide a major source of human infection, including risk through undercooked, contaminated poultry. *Campylobacter* spp. grow well at the body temperature of birds without causing illness in them. Most raw poultry meat is contaminated with *C. jejuni* (Chin, 2000). These pathogens are also commonly found in young cattle, swine, sheep, rodents, dogs and cats. Although they do not reproduce in waters at ambient temperatures, *Campylobacter* spp. are found in fresh and marine waters affected by birds or wildlife, domestic sewage and undisinfected sewage effluents; they survive best at colder temperatures (Blaser et al., 1983; Fricker, 1999). This group of pathogens is estimated to produce between 5 and 14% of all diarrheal disease worldwide (Chin, 2000).

Campylobacter produce acute gastroenteritis with diarrhea which may be either profuse and watery, or dysenteric, that is, containing blood and mucus. Diarrhea may be of variable severity and is often accompanied by abdominal pain (which may be severe enough to mimic appendicitis), headache, fever, nausea and vomiting. The disease appears with sudden onset, but may be preceded by flu-like symptoms and has a typical incubation period of 2 to 5 days within a range of 1 to 10 days, depending on the dose ingested (Fricker, 1999; Chin, 2000). The illness usually lasts 2 to 5 days, but may last more than 10 days. Illness may be prolonged in adults, and relapses may occur in up to 20% of cases (Blaser et al., 1983). Infected individuals who are not treated with antibiotics may shed organisms in feces for 2 to 7 weeks (Chin, 2000). Infection may be caused by ingestion of fewer than 500 of these pathogens. Immunity to specific strains is believed to develop after infection with those strains; in developing nations, immunity is generally acquired by age two.

In a small number of cases, a typhoid-like syndrome or reactive arthritis may occur and in rare cases, fever-related convulsions, Guillain-Barré syndrome (a paralysis that lasts several weeks and usually requires intensive care) or meningitis may occur. Chronic complications have been reported in 1 to 2% of cases (Fricker, 1999). Guillain-Barré syndrome is reported in only 0.1% of cases in the U.S., but the relatively high incidence of campylobacteriosis allows the possibility that 40% of all Guillain-Barré syndrome cases in the U.S. may be caused by campylobacteriosis. Some immunocompromised individuals may develop septicemia, a life-threatening condition. Overall, an estimated 100 fatalities may be caused in the U.S. each year by campylobacteriosis (CDC, 2003a).

A variety of culture techniques have been developed to isolate *Campylobacter*. Such techniques now typically involve sample concentration by filtration through a 0.22 µm filter, followed by pre-enrichment culture for 4 hours and then selective enrichment for 24 hours, followed by plating onto selective media for another 48 hours in a microaerobic environment with identification by staining, oxidase and catalase reactions and biochemical tests (Fricker, 1999). Serotyping for assessing human exposure to *Campylobacter* and PCR schemes for sensitive detection are also available, but the detection of viable pathogens remains a relatively complex and time-consuming procedure.

Conventional disinfection using chlorine is easily sufficient to inactivate *Campylobacter* to an adequate degree in drinking water supplies. These organisms appear to be somewhat more susceptible to chlorine than *E. coli*, and treated water systems that are maintained free of *E. coli* will also be free of *Campylobacter* (Fricker, 1999).

2.3.5 *Escherichia coli* (Enterohemorrhagic *E. coli*, Enterotoxigenic *E. coli*)

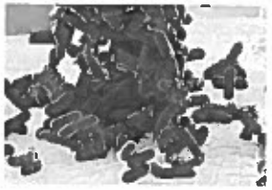

Photo by. G. Armstrong, University of Calgary

Escherichia coli is a member of the family *Enterobacteriaceae*. These bacteria are a vital component of the intestinal flora of warm-blooded animals (mammals and birds) because they assist in the digestion of food. However, at least six groups of pathogenic strains of these otherwise beneficial bacteria are now recognized: enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC); enterohemorrhagic *E. coli* (EHEC), which includes *E. coli* O157:H7; enteroaggregative *E. coli* (EaggEC); and diffuse adherent *E. coli* (DAEC). The ETEC and EHEC groups have been identified as causing major waterborne-disease outbreaks (Rice, 1999; Chin, 2000).

E. coli can survive with or without oxygen. They are gram-negative rods of 0.5 to 2.0 µm. They are motile by means of a flagellum and are unable to form spores to survive unfavourable environmental conditions, unlike a number of other bacteria. *E. coli* exhibit some characteristic metabolic capabilities that suit their role in assisting digestion and contribute to their identification: they produce acid and gas from lactose and indole from tryptophan.

The human gastrointestinal tract is the principal reservoir for all of the pathogenic *E. coli* strains, except EHEC, which has cattle as its primary reservoir (Rice, 1999). EHEC has also been identified in deer. The overwhelming majority of *E. coli* in the human gastrointestinal tract is non-pathogenic. Pathogenic strains of *E. coli* are spread by the fecal-oral route, with food or water contamination a primary cause of outbreaks, but secondary person-to-person transmission also occurs, in which case humans serve as a temporary reservoir (Chin, 2000).

E. coli are capable of surviving in aquatic and soil environments. Die-off is a function of temperature, nutrient levels, competing bacteria and solar radiation. In soil, dehydration may also play a role. Although research has been limited, pathogenic strains do not appear to differ in their survival rates from normal wild strains (Rice, 1999).

The symptoms of ETEC infection include a profuse, watery diarrhea with neither blood nor mucus, similar to that caused by cholera. Other symptoms include: abdominal cramping, vomiting, acidosis, extreme exhaustion and sometimes low-grade fever. ETEC strains produce either or both of two types of toxin: heat-labile toxins (one of which is closely related to cholera toxin) and heat-stable toxins (Hunter, 2003). The two groups who commonly experience this disease are young children in tropical countries, shortly after weaning (typically aged less than two), and non-immune adults (typically travelers from affluent countries). The latter may account for 20 to 40% of all cases of traveler's diarrhea, a condition affecting up to 60% of visitors to tropical countries (Hunter, 2003).

The symptoms of EHEC infection (most commonly *E. coli* O157:H7 in North America, Europe and Japan) include diarrhea that may range from mild and non-bloody to severe diarrhea that is virtually all blood. Serious diarrhea is accompanied by abdominal pain with little or no fever. Hemolytic uremic syndrome (HUS) may develop in 2 to 7% of cases with EHEC diarrhea. *E. coli* O157:H7 produces potent cytotoxins: the Shiga toxin(s), one of which is identical to the toxin produced by *Shigella dysenteriae*, which can also cause HUS (Chin, 2000). Fluid replacement is essential if these conditions are developing; however, antibiotics and antidiarrheals are not recommended because they may lead to kidney problems, which are especially serious for young children and the elderly. A follow-up study of 103 children who had HUS evaluated 88 and found a majority showed some biochemical evidence of residual kidney damage (Fitzpatrick et al., 1991).

The incubation time for ETEC is typically 1 to 3 days, but may be as short as 10 to 12 hours, with illness usually lasting fewer than 5 days. Excretion of ETEC and risk of person-to-person transfer may be prolonged (Chin, 2000). The incubation time for EHEC has a median of 3 to 4 days, but the observed range has been 2 to 8 days (Chin, 2000). The illness typically lasts about a week, but longer duration is possible (Rice, 1999). Complications like HUS will certainly lead to longer illness.

The median infective dose for ETEC is 10^8 to 10^{10} organisms, unless stomach acids are neutralized (e.g., extensive use of antacids), bringing the infective dose down to 10^6 organisms. The infective dose for EHEC is recognized to be much lower than for other toxic *E. coli* strains, although consensus is lacking on how low that infectious dose could be. One estimate for a median infectious EHEC dose is near 10^6 organisms (Haas et al., 2000), but another predicts infections being possible at about 10^2 organisms (Strachan et al., 2001).

The standard monitoring procedures for *E. coli* include ETEC. EHEC strains, including *E. coli* O157:H7, will be recovered in the total coliform assay at 35°C, but *E. coli* O157:H7 grows poorly at 44.5°C and is negative in tests for β-glucuronidase. These responses make the thermo-tolerant (fecal) coliform and routine *E. coli* assays unsuitable for isolating EHEC organisms. Additional tests

are needed to screen for EHEC types. Methods include sorbitol fermentation and glutamate decarboxylase tests or subculturing by growing on sorbitol-MacConkey (SMAC) agar. Recovery of these *E. coli* strains will be improved if large volumes of water are sampled and concentrated by membrane filtration or centrifugation, and then grown in enrichment media and identified with specific biochemical and serological tests (Rice, 1999). Molecular probes for toxin and virulence genes can provide definitive identification.

The pathogenic strains of *E. coli* are as susceptible to chlorination as non-pathogenic *E. coli* making them easy to disinfect under normal chlorination practices. A free chlorine dose of 0.2 mg/L with 1 minute contact time easily achieves a 99% kill of *E. coli* (Rice, 1999). An adequate disinfectant residual must be maintained in all areas of the water distribution system to offer any protection for re-contamination episodes.

2.3.6 *Salmonella*

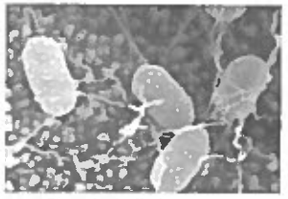

Photo by B. Finlay & S. Falkow, University of British Columbia

Salmonella species are members of a genus of the family *Enterobacteriaceae*, a large group of bacteria widely distributed in the environment (soil, water and animal wastes). More than 2000 *Salmonella* serotypes have been identified, but only about 200 are commonly encountered and their taxonomy is being revised according to current understanding of their DNA relationships (Covert, 1999; Chin, 2000). These bacteria prefer to live in the absence of oxygen; they do not form spores and are usually motile, gram-negative rods, 2 to 5 μm long and 0.8 to 1.5 μm wide.

Salmonellae are commonly found in animals such as poultry, swine, cattle, birds and rodents, and in pets, including turtles, iguanas, chicks, dogs and cats. Humans are also carriers, both when recovering from infection and during asymptomatic infection. Chronic carriers are common in animals, but are rare in humans (Covert, 1999). Transmission occurs through the fecal-oral route, mainly by ingestion of fecally contaminated and inadequately heated or disinfected food, milk and water.

Salmonellae have been reported to survive from 1 to more than 100 days (Feachem et al., 1983). Factors affecting survival include the presence of protozoa, organic matter, nutrients, ultraviolet (UV) light and temperature. *Salmonellae* have also been reported to survive for extended periods in contaminated surface waters, activated sludge effluents and other nutrient-rich waters (Covert, 1999).

Various forms of human disease are caused by pathogenic *Salmonellae* strains including gastroenteritis, enteric fever and blood poisoning. Gastroenteritis typically involves acute inflammation of the small intestine and colon accompanied by sudden onset of headache, abdominal pain, diarrhea, nausea and possibly vomiting. Some fever is commonly associated with this gastroenteritis, and dehydration from diarrhea can be severe if not adequately treated by fluid replacement (Chin, 2000). The predominant gastroenteric symptoms are caused by the pathogen invading only the surface layers of the gut. In some cases, deeper pathogen invasion occurs, allowing spread through the bloodstream, possibly leading to more serious conditions including blood poisoning, meningitis or abscess formation at remote sites (Hunter, 1997).

Typhoid fever is caused by *Salmonella typhi*, now proposed to be called *Salmonella enterica* serovar Typhi or simply *Salmonella* Typhi (Chin, 2000). Typhoid fever was once the most common form of waterborne disease in industrialized countries, occurring far more commonly than cholera. There are an estimated 17 million cases with 600,000 deaths worldwide annually, but fewer than 500 cases are reported in the U.S. and most of these cases are imported from endemic areas (Chin, 2000). Drinking water transmission of typhoid fever, like cholera, has been essentially eliminated from affluent nations, so this discussion will focus on *Salmonellae* pathogens causing outbreaks of gastroenteritis. Such outbreaks are, and should be, rare. However, a *Salmonella* gastroenteritis outbreak in Riverside, California in 1965 infected more than 16,000 people, hospitalized 70 and caused 3 deaths (Greenberg & Ongerth, 1966; Ross et al., 1966; Boring et al., 1971; Stone et al., 1971). This outbreak was followed by the Gideon, Missouri *Salmonella* gastroenteritis outbreak of 1993 (Section 4.5.14), which infected more than 600 and caused 7 deaths (Clark et al., 1996; Angulo et al., 1997).

The incubation period for *Salmonella* gastroenteritis ranges from 6 to 72 hours, but is most commonly 12 to 36 hours. Incubation periods will be shorter when higher pathogen doses are delivered. Bloody diarrhea may occur in up to 30% of cases, but the disease is usually self-limiting within 2 to 5 days. In unusual cases, illness may persist for weeks (Hunter, 1997; Covert, 1999).

A wide range of values has been reported for the median infective dose for non-typhoid salmonellosis, including estimates of 10^9 (Huner, 1997), 100,000 (Duncan & Edberg, 1995; Moe, 2001), below 1000 and possibly as low as 10 organisms (Hunter, 1997). This range suggests that the interplay of factors leading to infection is not fully understood. Infants and immunocompromised individuals are expected to be more susceptible.

In water, large samples must normally be concentrated by filtration, followed by selective enrichment to increase the ratio of *Salmonellae* to other bacteria likely to be present. *Salmonellae* positive isolates are confirmed with various commercial identification systems and serological testing (Covert, 1999). PCR and fluorescent-antibody techniques are now available, allowing for more sensitive environmental monitoring (Hunter, 1997). *Salmonellae* will be found

in surface waters wherever there are animal populations and are frequently found in wastewater effluents and receiving waters (Covert, 1999).

Chlorination inactivates *Salmonellae* as readily as *E. coli*, so maintaining adequate chlorination should achieve disinfection for *Salmonellae* (Covert, 1999).

2.3.7 *Shigella*

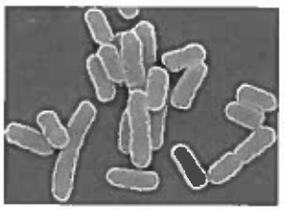

Photo by G. Tyrrell, M. Peppler & R. Sherburne, University of Alberta

Shigella species are a genus in the bacterial family *Enterobacteriaceae* with a number of species or serogroups: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C) and *S. sonnei* (serogroup D). Serogroup D has caused the majority of reported infections in the U.S. (Moyer, 1999). *Shigella* spp. prefer to live in the absence of oxygen. They do not form spores and are non-motile, gram-negative rods, 0.3 to 1 μm in diameter and 1 to 6 μm in length.

Humans are the only significant host, although outbreaks have occurred in primates. Shigellosis is transmitted by the fecal-oral route through contamination of water, milk and food (from sewage or sludge on croplands or from infected food-handlers). Person-to-person spread is caused by inadequate sanitation, poor hand-washing practices and poor personal hygiene.

Shigellae can survive outside the human host for up to 4 days in river water and for more than 44 to 100 days or longer in clean cold waters (Feachem et al., 1983; Hunter, 1997). They were found to die more slowly in well water at 9 to 12°C than fecal indicators; *Salmonellae* or *Vibrio cholerae* showed a half-life of about 24 hours (McFeters et al., 1974). *Shigellae* survive best in very clean, but unchlorinated, water or in polluted water that contains nutrients but few competitor bacteria (Feachem et al., 1983).

Shigellae cause bacillary dysentery, that is diarrhea that contains blood and mucus (Hunter, 1997). Other symptoms of shigellosis include: fever, nausea, vomiting, cramps and painful straining during attempted bowel movement. Shigellosis may range from mild, self-limiting diarrhea to much more severe symptoms including HUS and convulsions in young children (Chin, 2000). Worldwide, shigellosis is estimated to cause 600,000 deaths per year, with the majority of cases and deaths in children under ten.

The incubation time for shigellosis is usually 1 to 3 days, but may range from 12 to 96 hours with illness typically lasting 4 days to 2 weeks. The median infective dose is very low for a bacterial pathogen; as few as 10 to 200 bacteria may cause disease (Duncan & Edberg, 1995; Moyer, 1999; Chin, 2000; Moe, 2001).

As with other bacterial pathogens, *Shigellae* must be concentrated by membrane filtration or centrifugation, then enriched and grown on selective media followed by a variety of detection tests. More sensitive detection of organisms in environmental samples is possible with PCR (Moyer, 1999).

Shigella spp. are readily inactivated by chlorination. Drinking water outbreaks of shigellosis require both a source of human fecal contamination and inadequate chlorination or alternate disinfection (Hunter, 1997).

2.3.8 *Giardia lamblia* (syn. *G. intestinalis*, *G. duodenalis*)

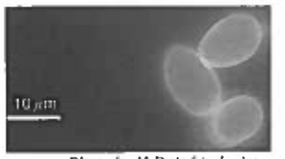

Photo by H.D.A. Lindquist, U.S. EPA

Giardia lamblia is the most widely used name for this common enteric protozoan parasite, but *G. intestinalis* has been proposed. *Giardia* is a unicellular, obligate parasite: it completes its life cycle within the small and large intestines of an animal host (Schaefer, 1999).

Giardia exists in two forms, a dormant, robust and infective cyst for transmission in the environment and a trophozoite, which is the active living form in the host gut. The round to oval cyst is 8 to 18 μm long and 5 to 15 μm wide containing a single organism with 4 nuclei. Once within a suitable host, the cyst releases a trophozoite that actively feeds, grows and reproduces. The trophozoite is 12 to 15 μm long and 6 to 8 μm wide with 2 nuclei, 4 pairs of flagella and a sucking disc to attach to the gut wall surface (Hunter, 1997; Schaefer, 1999).

Hosts for *Giardia* include pets such as cats, dogs, hamsters and gerbils; livestock such as sheep, cattle, horses and pigs; various wildlife such as beavers, muskrats and deer; and humans. Infection is spread by fecal-oral transmission including animal-to-human transmission, person-to-person transmission and fecal contamination of food and water. Trophozoites do not survive outside the host except in special media. Cysts can survive for months outside the host, particularly in cold water, but do not survive boiling or more than one cycle of freezing and thawing (Hunter, 1997; Schaefer, 1999; U.S. EPA, 1999b).

Giardiasis in humans may occur as an asymptomatic infection or as acute or chronic diarrhea. Other symptoms may include bloating, flatulence, cramps, loss of appetite, vomiting, weight loss, fatigue, mucus or blood in stool, malabsorption of ingested fats leading to pale, greasy and foul-smelling stool, malabsorption of fat-soluble vitamins and, occasionally, fever. Left untreated, symptoms may last from 10 days to 12 weeks or longer. Giardiasis is the most commonly reported intestinal protozoan parasite infection worldwide and prevalence surveys of infection among children range from 1 to 68% (U.S. EPA, 1999b; Chin, 2000).

Giardia has a relatively long incubation time of 7 to 10 days within a range of 1 to 75 days, depending on the ingested dose and health of the host. *Giardia* outbreaks typically have shown incubation periods of 1 to 3 weeks. When administered by ingestion of a gelatin capsule to healthy volunteers, as few as 10 cysts have been found to be infective, but a wide range of infectivity levels have been reported, suggesting differences in virulence among strains or other intervening factors that affect host susceptibility (Hunter, 1997; U.S. EPA, 1999b; Chin, 2000).

Monitoring and detection of *Giardia* cysts in water remains challenging because conventional culture techniques applicable to bacteria cannot be used. Cysts must first be concentrated from a large volume (10 to 100 L) of water by retention on specific filters with varying utility for different sample characteristics, followed by various schemes to elute, re-suspend and separate cysts from interfering particulate matter (Schaefer, 1999; U.S. EPA, 1999b). Recovery with reliable reporting of data has proven difficult (Allen et al., 2000). Immuno-fluorescence is used to detect *Giardia* cysts, which are examined under a microscope to judge their fluorescent colour, shape and internal structures. These techniques, however, cannot determine viability or infectivity (Schaefer, 1999; U.S. EPA, 1999b).

Giardia was the most commonly identified pathogen in waterborne outbreaks in the U.S. between 1971 and 1996, with 115 drinking water outbreaks causing 28,000 cases (Craun & Calderon, 1999). Surveys of wastewaters, receiving waters, source waters and treated drinking water have found that *Giardia* is often found in surface waters of North America (U.S. EPA, 1999b). As well, a large survey of U.S. groundwater sites found that 34% of sites susceptible to contamination were positive for *Giardia* or *Cryptosporidium*, or both, as were 14% of moderate-risk and 4% of low-risk sites (Moulton-Hancock et al., 2000).

Filtration with chemical coagulation and effective operation for consistent turbidity removal can reliably remove more than 99% of cysts from raw water, with optimal operations achieving 10- to 100-fold better removal (i.e., >99.99% overall removal). Chlorine can achieve more than 99% inactivation of cysts at lower pH and warmer temperatures, but very long contact times are needed to achieve substantial inactivation at higher pH and low temperatures. Chloramines are much less effective than free chlorine for inactivating *Giardia* (Schaefer, 1999; U.S. EPA, 1999b).

2.3.9 *Cryptosporidium parvum*

Cryptosporidium parvum is a coccidian protozoan and an obligatory parasite of the intestinal tract of warm-blooded animals. *C. parvum* is the one species of *Cryptosporidium* that produces human disease. This parasite was described

in 1912, but was first recognized as a cause of human disease in 1976. *Cryptosporidium* became prominent as a serious human pathogen following reports of severe diarrhea among AIDS patients in 1982 (Sterling & Marshall, 1999). Cryptosporidiosis was not generally recognized as a waterborne disease until after a 1984 outbreak in Braun Station, Texas (Section 4.4.11). Accordingly, this pathogen, which now dictates drinking water technology requirements, was not in the landmark 1983 World Bank report on Sanitation and Disease (Feachem et al., 1983).

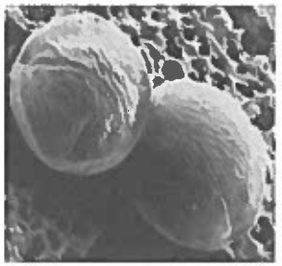

Photo by M. Belosevic, University of Alberta

Cryptosporidium is released in feces, is disseminated and survives in the environment as a robust, double-walled oocyst, 4 to 6 µm in size, which contains 4 sporozoites. Following ingestion, the oocyst releases the sporozoites into the small intestine. These invade the intestinal tract and each develop into trophozoites, then into merozoites which reproduce asexually, then into zygotes. Zygotes reproduce sexually to complete the cycle by forming oocysts, which are released back into the environment in the feces of the infected host (Hunter, 1997; Sterling & Marshall, 1999).

The normal hosts and reservoirs include humans, cattle and other domestic animals, birds and reptiles (Hunter, 1997; Sterling & Marshall, 1999). The young of most animal species are particularly prone to infection. Advances in molecular biology have allowed a distinction between *C. parvum* genotype 1, which apparently causes infection only in humans, and *C. parvum* genotype 2, which may cause infection in both animals and humans (Peng, et al., 1997).

Given their thick double wall, oocysts can survive for months in cold, moist environments, but are susceptible to heating and drying (Sterling & Marshall, 1999). Environmental monitoring surveys have shown that *Cryptosporidium* oocysts are commonly found in surface waters in the range from 0.01 to 100 per L (Fricker et al., 2002) and, as noted for *Giardia*, they are also found in groundwaters (Moulton-Hancock et al., 2000).

The main symptom of cryptosporidiosis in humans is profuse, watery diarrhea that may contain mucus or slime. Other symptoms include cramping, abdominal pain, nausea, vomiting (mainly in children), weight loss, mild fever and fatigue. Symptoms may reoccur after a period of recovery, but generally last less than 30 days. Prolonged and sometimes life-threatening infections may occur in people with immunodeficiency conditions, which are characterised by low numbers of T-cells (particular white blood cells). These groups essentially comprise people in the advanced stages of AIDS, people on intensive cancer chemotherapy and children with a congenital condition known as SCID (severe combined immuno-deficiency). Recent advances in drug therapies for HIV infection, which help to preserve immune function,

have greatly reduced the risk of severe cryptosporidiosis in this group (Ives et al., 2001). In addition, the first drug for treatment of cryptosporidiosis in children was approved by the US Food and Drug Administration in late 2002, and is undergoing trials in adult HIV patients. Thus, the status of cryptosporidiosis as an *untreatable* infection is changing.

The precise incubation time of cryptosporidiosis is unknown, but has been estimated to be 7 days within a range of 1 to 12 days (Chin, 2000; Fricker et al., 2002) or a range of 4 to 28 days (Hunter, 1997; Sterling & Marshall, 1999). The median infective dose was reported as 132 cysts in one volunteer study, but as few as 30 oocysts caused disease in one of five volunteers at that dose (Dupont et al., 1995). More recent and comprehensive volunteer studies have shown a wide range of infectious doses for different strains of *Cryptosporidium*, with the dose for a median risk of infection as low as 10 oocysts for one strain or as high as 1,000 oocysts for another, along with differences in attack rate and incubation time (Okhuysen et al., 1999). These differences help to explain the lack of a clear relationship between oocyst numbers in drinking water and the incidence of cryptosporidiosis observed in an outbreak.

As with *Giardia*, monitoring for *Cryptosporidium* requires sampling large volumes of water (10 to 100 L), which must be concentrated on a filter, eluted, centrifuged and collected on a membrane filter for staining with a fluorescent antibody. Identification under a microscope requires recognition of internal structures. Interference from fluorescent algae is a serious problem, and techniques to deal with this interference have not been entirely successful. Considerable effort has been directed towards improvement of monitoring techniques, but the methods used and the insight they provide remain limited, particularly because the methods commonly in use for water samples are unable to provide reliable information on the viability or infectivity of oocysts (Fricker et al., 2002).

Newly developed techniques combining the culture of oocysts in human cells with the detection of *Cryptosporidium* genetic material by PCR are able to provide an indication of viability and potential human infectivity, but are technically demanding and require sophisticated facilities (Rochelle et al., 1997; Slifko et al. 1997; DiGiovanni et al., 1999; LeChevallier et al., 2000; Rochelle et al., 2002). Variations on this method providing quantitation of oocysts have also been developed (Keegan et al., 2003).

Removal of oocysts by conventional water treatment processes requires optimization of coagulation, flocculation and rapid sand filtration performance for turbidity and particle removal to achieve consistent filter effluent turbidity of less than 0.1 nephelometric turbidity unit (NTU). Under optimal conditions, 99 to 99.9% of oocysts may be removed (Fricker et al., 2002). Oocysts are very resistant to chlorine disinfection, making this process an inadequate barrier to *Cryptosporidium*. The main breakthrough in disinfection practice has been the discovery that low dosages of UV will damage, but not kill, the oocysts, rendering them unable to reproduce within a host (Clancy et al., 2000).

2.3.10 *Toxoplasma gondii*

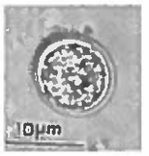

Photo courtesy of J.P. Dubey et al., 1998

Toxoplasma gondii is an obligate protozoan parasite that survives and is transmitted through the environment (water and soil) in the form of oocysts that are 10 to 12 µm in size (Dubey, 1999). Transmission occurs from consumption of infected animal tissue, by consumption of food or water contaminated by feces from infected cats or through the placenta in pregnancy.

In the intestine of an animal that has ingested oocyst-contaminated food or water, one oocyst releases eight sporozoites, which multiply in the intestinal tract and associated lymph nodes, forming tachyzoites, which are rapidly reproducing forms that migrate throughout the body via the blood and lymph systems. These tachyzoites typically form cysts in the skeletal or heart muscles, the brain and the liver, and remain there, viable but inactive and otherwise asymptomatic for the duration of the host's life. *T. gondii* can be transmitted congenitally to the fetus during early pregnancy, potentially causing hearing impairment, vision loss, mental retardation or fetal death. Infection later in the pregnancy usually causes no symptoms or milder symptoms, including lesions or irritation of the eyes (Chin, 2000). In the case of immunocompromised individuals, particularly those with AIDS, these cysts can cause overwhelming disease, with encephalitis as a predominant feature (Dubey, 1999).

Cats, both domestic and wild, are the only known hosts of *T. gondii* (Dubey, 1999) and only cats shed oocysts in their feces. Cats become infected when they eat birds or animals infected with dormant tissue cysts, which are then re-activated in the cat and ultimately shed as oocysts in its feces. Oocysts shed by cats become infective between 1 to 5 days after defecation (Chin, 2000). Feral cats are more prone to carrying *T. gondii* than domestic cats because feral cats consume infected birds and animals in the wild (Dubey, 1999). *T. gondii* can infect essentially all warm-blooded hosts and causes widespread infection in humans worldwide.

The incubation period of oocysts after ingestion has been reported to be between 5 and 23 days (Chin, 2000). Oocysts can be sampled with techniques used for concentrating other protozoan cysts and oocysts (Isaac-Renton et al., 1998). Although oocysts are readily seen by microscope, currently they must be confirmed by infecting mice because they look so similar to other protozoan oocysts. The oocysts are extremely resistant to environmental conditions, including freezing, drying and heating up to 56°C. Disinfection

requirements (CT values) are not reported for *T. gondii* oocysts, but the oocysts are considered highly resistant to chlorine (Dubey, 1999). Given their size, these oocysts should be removed by water treatment processes somewhat better than *Cryptosporidium* oocysts, likely similarly to *Giardia* cysts, but specific treatment studies on removal of *T. gondii* oocysts have not been reported.

2.4 INDICATOR AND SURROGATE ORGANISMS

Although specific pathogens have been identified from the earliest days of microbiology, the laboratory methods required for such identification are challenging and often complex. The presence of pathogens in treated water is expected to be a rare event unless the source water is highly polluted and the treatment is unable to cope with the raw water challenge. Even methods that detect the presence of hazards accurately will be challenged for monitoring purposes if the hazard to be detected occurs only rarely (Hrudey & Leiss, 2003). After the connection between fecal contamination and the risk of waterborne disease was established, monitoring for indicator organisms that are easier to detect became an accepted practice (Pipes, 1982; Payment et al., 2003). Desirable characteristics for an indicator organism have been proposed (Bonde, 1966; Olivieri, 1982). The indicator should:

1. be present when the pathogenic microorganisms of concern are present but should be absent in uncontaminated water;
2. be present in numbers much greater than the pathogen or pathogens it is intended to indicate;
3. respond to environmental conditions and water and wastewater treatment processes in a manner similar to the pathogens of interest;
4. be easy to isolate, identify and enumerate.

E. coli was recognized to be the most prevalent bacteria in human feces, making it an indicator for the presence of human feces and associated enteric pathogens. However, the lack of a simple one-step test for *E. coli* led to the total coliform and then the fecal (thermo-tolerant) coliform groups being used as surrogate measures (Edberg et al., 2000; Health Canada, 2002; Payment et al., 2003). In turn, these surrogates were adopted as indicators for fecal pathogens, even though neither is truly indicative of the presence of *E. coli*, nor is *E. coli* a perfect indicator for all waterborne pathogens.

More recently, the utility of the total coliform measure has been called into question as an indicator of public health risk associated with fecal contamination of drinking water (Stevens et al., 2001; Health Canada, 2002; Payment et al., 2003). A study of the U.S. Total Coliform Rule for its ability

to predict vulnerability to outbreaks found that total coliform monitoring requirements were inadequate to identify systems vulnerable to an oubreak (Nwachuku et al., 2002). The primary criticism of the total coliform measure is that unless the measure is made on water that is grossly contaminated with fecal material, the majority of culturable organisms are natural flora that are not of fecal origin. This criticism is also true for the thermo-tolerant coliforms (previously, but inaccurately, called fecal coliforms), some of which are natural (non-fecal) flora of water. Consequently, the detection of *E. coli* now tends to be the preferred indicator of potential fecal contamination.

Some qualifiers need to be considered even if specific detection of *E. coli* is adopted. Effectively treated and disinfected drinking water that is fully protected from fecal re-contamination should be essentially free of *E. coli*. However, that absence cannot assure the absence of fecal pathogens that are more resistant to treatment or disinfection, such as some viruses and the protozoan pathogens *Giardia*, *Cryptosporidium* and *T. gondii*. For the latter, treatment-related specifications are used such as maintaining consistently low turbidity in treated water. Other microorganisms have been proposed as suitable indicators of treatment efficacy such as clostridial spores, aerobic spore-formers, total plate count and bacteriophages, but there is currently no consensus on their use (except for aerobic spore-formers) (Payment et al., 2003).

Given the limitations associated with relying on indicator organisms to protect against pathogens in drinking water, direct monitoring for pathogens has been advocated. Recently, in lieu of monitoring for other microbial or physico-chemical indicators, the continuous monitoring for *Cryptosporidium* oocysts has been mandated under the Water Supply (Water Quality) Regulations governed by the Drinking Water Inspectorate for England and Wales (DWI, 2000). This regulation was implemented in part because of an unsuccessful prosecution of a water company following an outbreak of cryptosporidiosis (South Devon–Torbay case study, Section 4.5.20). In that case, the court ruled that epidemiological evidence of the outbreak was hearsay, making it inadmissible as proof of health consequences. Consequently, regulations now oblige treatment plants that are judged by a specified risk assessment process to be challenged by *Cryptosporidium* oocysts in their source water to monitor their treated water continuously at a rate of at least 40 L/h. These regulations make it a criminal offence for these suppliers to exceed 1 oocyst per 10 L in their treated water. Because this is an operational treatment standard rather than a means of public health surveillance, guidelines have been developed to instruct public health authorities about how to respond appropriately if they are notified of an excess oocyst detection by a water company (Hunter, 2000).

Direct pathogen monitoring in treated drinking water has been strongly criticized. Critics have called the pathogen monitoring approach "*a pretense for public health protection*" maintaining that "*pathogen monitoring is of little value and should be replaced by alternative strategies such as treatment*

optimization" and that "*A more realistic approach to achieving public health protection is through source water protection, treatment optimization, and maintenance of water quality through storage and distribution.*" (Allen et al., 2000). Pathogen monitoring in treated water has also been challenged on the grounds of questionable public health efficacy (Fairley et al., 1999). Critics argue that, given the large number of pathogens, there is little value in attempting to measure all of them and that the absence of any of them does not guarantee risk-free water. Furthermore, detecting the presence of a pathogen without the presence of overt disease does not necessarily inform as to what levels of pathogen are tolerable or may be infective.

Another aspect of this debate is the recognition that microbial monitoring as it is now constituted, with discrete grab samples and product testing, is woefully ill-equipped to catch the inherently non-homogeneous nature of pathogen contamination of drinking water (Gale & Stanfield, 2000). As will become obvious in Chapter 4, the factors contributing to outbreaks are mainly intermittent events. This problem is amplified by evidence that water treatment processes such as coagulation tend to cause aggregation of pathogens, distributing them unevenly in treated water. Such evidence raises the plausible scenario of many consumers drinking glasses of water completely free of pathogens with only the occasional unlucky consumer drinking a glass containing an infective cluster of pathogens (Gale et al., 1997). Even untreated or lightly treated drinking water from sources presumed to be of high quality can face this problem because of the potential for clustering of pathogens within small particles of fecal origin (Schoenen, 2002). Anything other than continuous sampling, as has been mandated for *Cryptosporidium* by the Drinking Water Inspectorate for England and Wales, will tend to underestimate the potential for disease transmission, regardless of whether indicators, surrogates or pathogens are the target of the monitoring program.

While the arguments for and against specific pathogen monitoring are likely to continue for some time, the insights about the occurrence of oocysts in the water supplies of England and Wales since the introduction of the monitoring regulation are certainly interesting (Drury & Lloyd, 2002). Using 19 months of data from April 1, 2000 to December 1, 2001, 77,727 samples taken at 171 sites were analyzed. Oocysts were detected in 8.9% of samples with ~90% of detections in the range of 0.01 to 0.10 oocysts per 10 L. During this period, seven results (0.01%) contravened the standard with the highest result being 4.91 oocysts per 10 L. Individual plant performance ranged from no oocysts detected in 644 samples to detections in 77% of 627 samples in one system.

These issues and a detailed examination of the utility of various parameters for a wide range of water quality management purposes have been elaborated

in a recent international monograph on microbial safety of drinking water (Dufour et al., 2003). Some of these issues will be revisited in Chapter 6. Perhaps, given the benefit of the hindsight that we now have over the "*miasma*" versus "*contagion*" theories of cholera transmission, the current debates over the value of pathogen monitoring will one day be resolved by recognizing some merit in both sides of the debate.

2.5 SUMMARY OF PATHOGENS

The study of waterborne pathogens can become exceedingly complex and the foregoing discussion of them has been kept intentionally brief. Some of the problems discussed in Chapter 4 have arisen because inadequate attention has been paid to the larger issue: that waterborne pathogens are found with fecal wastes, either human or animal. Where sanitation levels are generally high, preventing waterborne disease is conceptually simple: keep the pathogens, which are inevitably found in the ever-present waste sources, from being delivered in drinking water at concentrations high enough to be infective.

Table 2.3 summarizes some of the salient features of the waterborne pathogens reviewed in this section (WHO, 2004). This table also includes a number of pathogens that are not reviewed in this book, but that are certainly relevant to waterborne disease in various parts of the world. The extract from the WHO table does not include the helminth parasites that cause millions of cases of debilitating disease around the world, but which have not posed any recent problems for drinking water supplies in affluent nations.

An indication of the likelihood of infection from ingestion of a single pathogen is estimated in Table 2.4, derived from Hurst (2002). Finally, to provide some perspective and context for the relative severity of disease caused by the various waterborne pathogens, data from various sources has been used to extend the table developed by Mead et al. (1999) in the summary provided with Table 2.5.

Having considered the sources of hazard to drinking water, the responses necessary to achieve safe drinking water must be understood, in particular, their strengths and weaknesses. The characteristics and capabilities of interventions (barriers) to assure safe drinking water are reviewed briefly in Chapter 3.

Table 2.3 Waterborne pathogens and their significance in water supplies (extracted from WHO, 2004, with permission)

Pathogen	Health significance	Persistence in water supplies[a]	Resistance to chlorine[b]	Relative infectivity[c]	Important animal source
Bacteria					
Burkholderia pseudomallei	Low	May multiply	Low	Low	No
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate	Yes
Escherichia coli – Pathogenic[d]	High	Moderate	Low	Low	Yes
E. coli – Enterohemorrhagic	High	Moderate	Low	High	Yes
Legionella spp.	High	Multiply	Low	Moderate	No
Non-tuberculous mycobacteria	Low	Multiply	High	Low	No
Pseudomonas aeruginosa[e]	Moderate	May multiply	Moderate	Low	No
Salmonella typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	Low	No
Yersinia enterocolitica	High	Long	Low	Low	Yes
Viruses					
Adenoviruses	High	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A	High	Long	Moderate	High	No
Hepatitis E	High	Long	Moderate	High	Potentially
Noroviruses and Sapoviruses	High	Long	Moderate	High	Potentially
Rotavirus	High	Long	Moderate	High	No
Protozoa					
Acanthamoeba spp.	High	Long	High	High	No
Cryptosporidium parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia lamblia	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply[f]	High	High	No
Toxoplasma gondii	High	Long	High	High	Yes

Note: Waterborne transmission of the pathogens listed has been confirmed by epidemiological studies and case histories. Part of the demonstration of pathogenicity involves reproducing the disease in suitable hosts. Experimental studies in which volunteers are exposed to known numbers of pathogens provide relative information. As most studies are done with healthy adult volunteers, such data are applicable to only a part of the exposed population, and extrapolation to more sensitive groups is an issue that remains to be studied in more detail.

a Detection period for infective stage in water at 20°C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month.
b When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed.
c From experiments with human volunteers or from epidemiological evidence.
d Includes enteropathogenic, enterotoxigenic and enteroinvasive.
e Main route of infection is by skin contact, but can infect immunosuppressed or cancer patients orally.
f In warm water.

Table 2.4 Probability that a human will become infected by ingesting a single microbial pathogen[a] (adapted from Hurst, 2002)

Type of microorganism	Probability of infection per ingested microorganism[e]
Viruses[b]	
Enteric pathogenic viruses (overall estimate)	0.5
Rotavirus	~1[f]
Bacteria[c]	
Enteric pathogenic bacteria (overall estimate)	0.00001
Protozoa[d]	
Cryptosporidium parvum	0.033
Giardia lamblia	0.1

a Probabilities were determined by volunteer feeding studies. The success of infection was determined by testing the sera of the volunteers before and after those individuals were dosed with microorganisms. The values listed in this table are medians based on data published by Hurst et al (1996). When values for the same genus or species of microorganism were available from more than a single study, an overall estimate was derived to represent that genus or species by calculating the median of the pertinent values. Likewise, overall estimates for any particular group (e.g., enteric pathogenic bacteria) of microorganisms were derived by calculating the median of the values available from studies in which members of that group had been examined.
b The number of viruses was determined by infectivity assay in cultured cells.
c The number of bacterial organisms was determined by culture.
d The number of protozoa was determined as either cysts (for *Giardia*) or oocysts (for *Cryptosporidium*) by direct microscopic enumeration.
e Probability of infection associated with each microorganism ingested. This calculation is performed as 1/minimum infectious dose.
f For this virus type, the number of virus particles required to cause an infection of cultured cells is greater than the number of virus particles required to cause infection of a human. Thus, the value of the probability of a human developing an infection from this virus type is higher than the titer obtained by cell culture assay of the virus.

Table 2.5 Estimated health effects of foodborne pathogens in the United States for those that are also waterborne (Sources: Mead et al., 1999; Chin, 2000; Haas et al., 1999; Marshall et al., 1999; Hurst, 2002; Moe, 2002)

Pathogen	Fecal Source	Incubation period (days)	Illness duration (days)	Total annual cases in U.S. (est.)	Fraction of cases foodborne[a] (%)	Hospitalization rate (%) (Mead et al. 1999 unless indicated)	Chronic conditions that may follow acute infection	Case fatality rate (%)
Bacteria								
Campylobacter jejuni	human or animal	1 to 10	2 to 5	2.4 million	80	3[b] - 10	reactive arthritis, Guillain-Barré syndrome	0.1
Escherichia coli O157:H7	human or animal	3 to 8	1 to 12	73,000	85	13[b] - 30	hemolytic uremic syndrome (HUS), kidney damage	0.8
Escherichia coli enterotoxigenic	human	0.5 to 5	3 to 5	79,000	70	0.5		0.01
Salmonella non-typhoidal	human or animal	0.3 to 3	2 to 5	1.4 million	95	4[b] - 22	reactive arthritis, meningitis, endocarditis, pneumonia, osteomyelitis	0.8
Shigellae	human	0.5 to 7	4 to 14	450,000	20	6[b] - 14	reactive arthritis, HUS kidney damage	0.2
Viruses								
Norovirus	human	1 to 3	0.5 to 4	23 million	40	n.e.[c]		n.e.[c]
Rotavirus	human	1 to 3	3 to 7	3.9 million	1	n.e.[c]		0.55[d]
Hepatitis A	human	15 to 50	7 to months	83,000	5	13 – 28[d]	reversible liver damage	0.1 to 0.3

Pathogen	Fecal Source	Incubation period (days)	Illness duration (days)	Total annual cases in U.S. (est.)	Fraction of cases foodborne[a] (%)	Hospitalization rate (%) (Mead et al. 1999 unless indicated)	Chronic conditions that may follow acute infection	Case fatality rate (%)
Protozoa								
Cryptosporidium parvum	human or animal	4 to 28	4 to 30	300,000	10	1[d] - 15		0.5
Giardia lamblia	human or animal	5 to 25	7 to >100	2 million	10	0.5[b]	lactose intolerance, chronic joint pain	n.e.[c]
Toxoplasma gondii	animal or meat	5 to 23	n.a.[e]	225,000	50	n.e.[c]	mental retardation, loss of vision, hearing impairment	n.e.[c]

[a] Waterborne disease fraction would be some small portion of 100% minus the estimated foodborne fraction

[b] estimates from (Haas et al., 1999)

[c] n.e. = not estimated; the methodology used by Mead et al. did not allow estimates of hospitalization rate and case fatality rate to be estimated for these pathogens and no other estimates were found

[d] estimates from (Hurst, 2002)

[e] n.a. = not applicable; toxoplasmosis has an ill-defined duration because cysts of *T. gondii* can remain dormant in tissue for an entire lifetime

Safe Drinking Water

Lessons from Recent Outbreaks in Affluent Nations

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